REACTIONS OF CARBON WITH ATOMIC GASES

By J. D. Blackwood* and F. K. McTaggart†

[*Manuscript received July* 30, 1959]

Summary

Wood chars were reacted at atmospheric temperature with hydrogen atoms, oxygen atoms and carbon monoxide, hydrogen atoms and hydroxyl radicals, produced by the action of a radio frequency field on hydrogen, carbon dioxide, and water vapour respectively. The chars were prepared at different temperatures and contained different amounts of oxygen. The experimental results showed that the gases must be present in the atomic form before reaction with the carbon can take place and that such species react on the carbon-surface independently of active sites.

In normal gasification processes the atomic species appear to be produced at active centres, which for the chars used could be correlated with specific oxygen groups remaining in the carbon. It is suggested that these groupings may have a pyran structure. An explanation has been put forward for the retardation of the carbon–water vapour reaction by hydrogen, and of the carbon–carbon dioxide reaction by carbon monoxide. These are considered as due to reverse mechanisms which decrease the concentration of the atomic species and not to the blocking of active sites by adsorption of the retardant.

I. Introduction

It has been shown (Blackwood and McTaggart 1959) that atomic oxygen, produced by the action of a radio frequency (R.F.) field, will react readily with amorphous carbon at ambient room temperature. This experimental approach produced much useful information on the fundamental process of oxidation. It is known that other common gasifying media such as hydrogen (Broida and Gaydon 1953), carbon dioxide (Sihvonen 1933), and water vapour (Rodebusch, Wende, and Campbell 1937 ; Broida and Kane 1953) can also be decomposed by the action of an electric discharge or R.F. field into products such as atomic hydrogen, carbon monoxide, and atomic oxygen, or atomic hydrogen and hydroxyl radicals respectively. It was thought that these products might react with carbon at atmospheric temperature, and this led to the possibility of obtaining information on the mechanisms of attack of carbon by these gases, processes which, until now, have not been fully understood. This possibility was therefore examined experimentally and the results are described below.

II. Experimental

(*a*) *Apparatus*

The apparatus used in this work has been described in a previous paper (Blackwood and McTaggart 1959). Some modification was made to the R.F. power generator by using a type 100TH thermionic valve as the amplifier.

* Chemical Engineering Section, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

† Division of Mineral Chemistry, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

(*b*) *Materials*

The gases used were from cylinders and contained only nitrogen as a significant impurity. Water vapour was obtained by evaporation of freshly distilled water under controlled temperature to produce the required vapour pressure.

The carbon samples used in this study were prepared by carbonizing jarrah wood (*Eucalyptus marginata*) in a stream of dry, oxygen-free nitrogen to a temperature of 650 °C. The resultant char was crushed and sifted and the fraction −7 +14 B.S. sieve collected. This material was blended and divided into six portions and each portion heated in dry, oxygen-free nitrogen to a given temperature and then allowed to cool to atmospheric temperature with nitrogen still flowing. The temperatures used were 650, 750, 850, 950, 1050, and 1150 °C. The resultant chars were analysed for carbon, nitrogen, hydrogen, and ash by the standard methods.

Oxygen was determined as described by Blackwood (1959) and is expressed as oxygen liberated as carbon dioxide and carbon monoxide when the carbon sample is heated in dry, oxygen-free nitrogen at a temperature of 1250 °C. The analyses and properties of the samples are shown in Table 1.

TABLE 1

ANALYSES AND PROPERTIES OF SAMPLES

Temperature of Preparation (°C)	C (%)*	H (%)*	N (%)*	Oxygen Liberated as		Oxygen as CO Liberated after Reaction with		Ash (%)
				CO_2 (%)	CO (%)	H_2 (%)	CO_2 (%)	
650	92·55	2·69	0·10	0·47	2·56	0·55	1·70	0·05
750	94·24	1·70	0·11	0·39	1·27	0·51	1·17	0·02
850	96·00	1·46	0·11	0·31	0·73	0·30	0·71	0·11
950	96·43	0·99	0·10	0·37	0·61	0·31	0·58	0·07
1050	97·48	0·68	0·09	0·22	0·41	0·20	0·44	0·06
1150	97·42	0·69	0·08	0·27	0·34	0·19	0·32	0·08

* By weight.

(*c*) *Procedure*

A sample of 0·5–0·75 g of the required carbon contained in a silica boat was placed in the reaction tube at a position sufficiently far from the R.F. field to prevent back diffusion and entry into the field of the products of reaction. The apparatus was then evacuated and the gas flow started. The greater part of the dead space in the vacuum pump was filled with oil and sufficient time was allowed for the purging of the apparatus, and the small residual dead space in the pump. The R.F. field was then applied and the reaction commenced. The gas collection rate, 250–350 c.c./hr, and pressure, 0·3–0·5 mm Hg, were measured and after suitable sweep times samples of the exit gas were taken. Analyses of these gases were made using either a gas chromatograph with silica

gel column and hydrogen carrier gas or with a Bone and Wheeler gas analysis apparatus. The rates are expressed as gram moles of product per minute per gram of carbon originally present.

III. Results

The reactions of carbon with the following gases were studied.

(*a*) *Water Vapour*

Water vapour at a controlled pressure was passed through the R.F. field and the products passed over the carbon sample. Reaction occurred as indicated by the change in colour of the glow along the tube from red to bluish white.

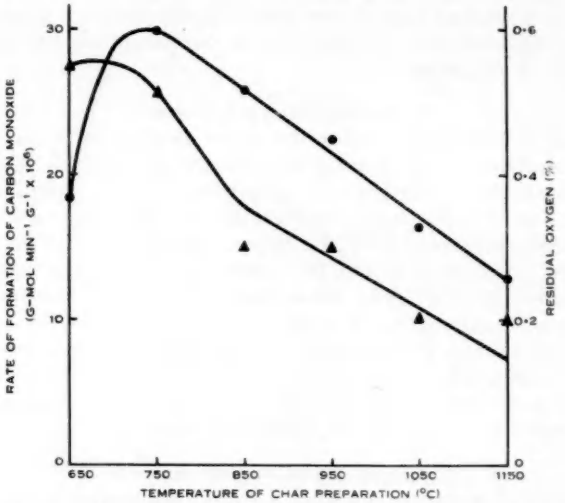


Fig. 1.—Relationship between temperature of preparation of chars.
● Reaction rate to dissociated water vapour.
▲ Residual oxygen content.

The product gases contained hydrogen, carbon monoxide, oxygen, and carbon dioxide. Although it has been stated that thermal decomposition (Tsuchuja 1954), electrical discharges, and R.F. fields (Rodebusch, Wende, and Campbell 1937; Broida and Kane 1953; Saunders *et al.* 1954) all decompose water into hydrogen atoms and hydroxyl radicals, it was thought desirable to examine spectroscopically the species present after passage of water vapour through the field. The results of these experiments showed that the reactant gases consisted mostly of hydrogen atoms and hydroxyl radicals and that there was little atomic oxygen present. In the absence of carbon the gases collected consisted entirely of oxygen and hydrogen and represented at least 90 per cent. dissociation of the original water vapour and were in the same stoichiometric ratio as in water.

With the different samples of carbon, the product was almost entirely carbon monoxide and the rate of production was a function of the temperature of

preparation of the carbon. Figure 1 shows the relationship of charring temperature to the rate of production of carbon monoxide, the pressure being the same in all cases.

For a given carbon, the rate of formation of carbon monoxide from dissociated water vapour was lower than for a comparable partial pressure of oxygen alone. For example, the 750 °C char gave a reaction rate of 50×10^{-6} g-mol min^{-1} g^{-1} when reacted with oxygen, compared with 30×10^{-6} g-mol min^{-1} g^{-1} when reacted with dissociated water vapour, the partial pressures of oxygen calculated as O_2 being the same in each case. In the case of simple oxidation all the atomic oxygen was consumed (Blackwood and McTaggart 1959) and the rate was dependent on the rate of supply of atomic oxygen, whereas, with water vapour, the rate was dependent also on the nature of the carbon. Long and Sykes (1948) have suggested that the rate may be dependent also on the inhibiting action of one of the products.

(*b*) *Hydrogen–Oxygen Mixtures*

In order to determine whether the slower reaction of water vapour and change of reactivity with charring temperature was due to retardation by hydrogen, mixtures of hydrogen and oxygen in the same stoichiometric ratio as in water vapour were passed through the R.F. field and over the carbon samples. Under these conditions, the carbon monoxide rate for the 650 °C char was 52×10^{-6} compared with 47×10^{-6} g-mol min^{-1} g^{-1} using oxygen only and $18{\cdot}5\times10^{-6}$ g-mol min^{-1} g^{-1} with dissociated water vapour, the oxygen partial pressure being comparable in all cases. This indicates that the rate was not reduced by the presence of hydrogen. In each case, 89 per cent. of the product gases on a hydrogen-free basis consisted of oxides of carbon. All the chars behaved similarly giving approximately the same carbon monoxide rate of 50×10^{-6} g-mol min^{-1} g^{-1} under the same conditions.

(*c*) *Oxygen–Water Vapour Mixtures*

The above results made it appear unlikely that hydrogen was responsible for the inhibition of oxidation of carbon by water vapour. Since the breakdown of water vapour in the R.F. field was not complete, it was considered desirable to determine if the undecomposed water vapour was responsible. Accordingly, oxygen was passed through the apparatus and the atomic oxygen produced was allowed to react with a carbon sample. Water vapour was then introduced into the apparatus close to, and on the upstream side, of the carbon sample but at such a position that it could not diffuse back into the field. A blank experiment in which no carbon was present, showed that about 5 per cent. of the water vapour was dissociated into hydrogen and oxygen by the action of the atomic oxygen, and hence 95 per cent. of the water vapour introduced was present as water molecules.

When a sample of the 1150 °C char was reacted in the apparatus, with oxygen alone, the rate was 54×10^{-6} compared with 48×10^{-6} g-mol min^{-1} g^{-1} when water vapour was admitted, at constant oxygen partial pressure. The change in rate was not significant and was considerably higher than the value of 13×10^{-6} g-mol min^{-1} g^{-1} obtained with dissociated water vapour.

(*d*) *Hydrogen*

Since it was known that the high temperature reactivity of carbon to molecular hydrogen (Blackwood 1959) and to dissociated water vapour (see above) was a function of the temperature of preparation of the carbon and probably of specific oxygen groupings, it was of interest to observe whether hydrogen in the atomic state would show the same relationship.

Hydrogen at a controlled pressure was passed through the R.F. field and the resultant atomic hydrogen allowed to react with the carbon samples. The main product of the reaction was methane, although initially a small amount of

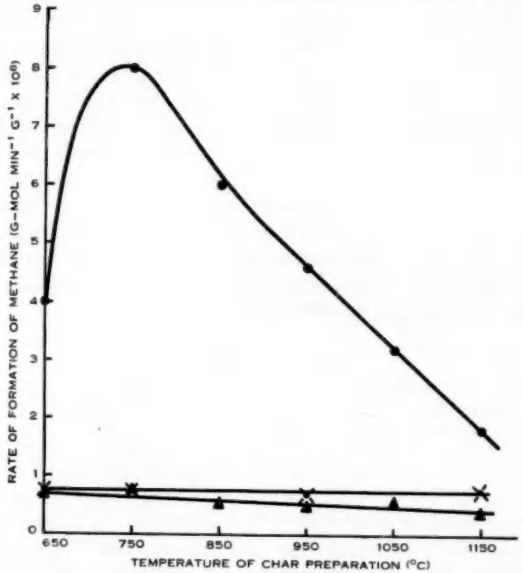


Fig. 2.—Showing difference in reactivity of chars, prepared at different temperatures, to hydrogen.

● Molecular hydrogen at 650 °C and 30 atm.

× Atomic hydrogen at room temperature and 0·3 mm Hg on hydrogen pretreated chars.

▲ Atomic hydrogen under the same conditions on carbon dioxide pretreated chars.

carbon monoxide was also formed. There did not appear to be any significant change in reaction rate with change in temperature of charring. The 750 °C char gave a methane rate of $0{\cdot}62\times10^{-6}$ compared with $0{\cdot}59\times10^{-6}$ g-mol min^{-1} g^{-1} for the 1150 °C char. It was thought that in these experiments some methane might have diffused back into the field and decomposed, thus tending to mask the effect of change of reactivity since experiments with methane had already shown that this gas is decomposed in an R.F. field. However, when the tube length was increased so that back diffusion was negligible, the rates were 0·80

and 0·76 g-mol min^{-1} g^{-1} respectively, for the 750 and 1150 °C chars, showing that although some methane had been decomposed in the previous experiments, the uniformity in reactivity was still evident.

It has been observed (Blackwood 1959) that when molecular hydrogen reacts with carbon at high hydrogen pressures, a rapid initial reaction occurs in which a considerable amount of methane and water is formed. After a certain proportion of the oxygen in the carbon sample has been eliminated, the rate of methane formation becomes relatively steady and the reactivity measured at this stage is a function of the temperature of preparation of the char. If this reaction were to take place during the hydrogenation of carbon with atomic hydrogen, it might well mask the effect of change in reactivity with temperature of char preparation. In order to test this possibility, samples of chars were pretreated at 650 °C and 30 atm pressure with hydrogen and the rate of methane formation observed. When the observed rate had reached a steady value, the apparatus was cooled and the samples recovered. A similar set of samples was treated in the same manner with carbon dioxide. The residual oxygen contents for these two sets of samples are given in Table 1. These treated samples were then reacted with atomic hydrogen and the rates of methane formation measured. The results are shown in Figure 2, and it can be seen that there was no evidence of the behaviour shown by dissociated water vapour as in (*a*) above or by molecular hydrogen, although there is some indication of a slight decrease in reactivity with increasing temperature of char preparation.

(*e*) *Carbon Dioxide*

Carbon dioxide was passed through the R.F. field at a known pressure and the products allowed to react with carbon. With the power input used in these experiments, the carbon dioxide was split to carbon monoxide and atomic oxygen and the resulting oxygen reacted with the carbon to produce carbon monoxide. The reaction with the atomic oxygen produced was complete and chars showed no change in reactivity with temperature of preparation.

It has been observed (Blackwood and Ingeme 1960) that when carbon is gasified with carbon dioxide under pressure at high temperatures in the absence of an R.F. field, there is a rapid decrease in reaction rate over the first 10 to 20 min of reaction, similar to the effect observed during hydrogenation. To ensure that this effect was eliminated, samples were used which had been pretreated as described in (*d*) above. The samples thus treated showed no change in reactivity to carbon monoxide–atomic oxygen mixtures, although there was a considerable change of reactivity to molecular carbon dioxide at 650 °C and 1·2 atm pressure, which was again related to specific oxygen contents of the carbon. These results are shown in Figure 3.

Since the retarding effect of hydrogen in the carbon–steam system has been suggested as due to the occupation of active sites (Long and Sykes 1948) while carbon monoxide has no specific effect, it would be expected that, if the mechanism of the carbon–carbon dioxide reaction were similar, hydrogen would have a noticeable retarding effect on this latter reaction. Experiments were made using chars prepared at three different temperatures, in which the partial pressure

of carbon dioxide was maintained constant and hydrogen was introduced. A similar experiment was made replacing the hydrogen with nitrogen. The results, shown in Table 2, indicate that the amount of carbon monoxide produced was not substantially changed.

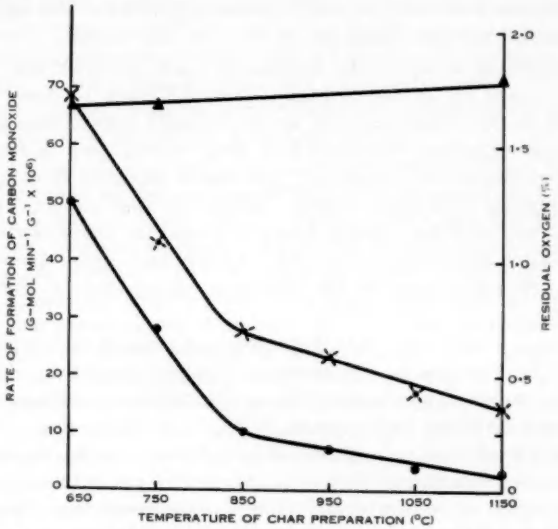


Fig. 3.—Relationship between temperature of preparation of char, reactivity to carbon dioxide and residual oxygen content.
● Molecular CO_2 at 650 °C and 1·2 atm.
× Residual oxygen content.
▲ Dissociated CO_2 at room temperature and 0·3 mm Hg.

In these experiments the partial pressure of carbon dioxide was approximately 0·3 mm Hg, comparable with the pressure of water vapour used in the water vapour–carbon experiments. The rates were generally higher for the

TABLE 2
RATE OF FORMATION OF CARBON MONOXIDE

Sample Char	Rate of CO Formation ($\times 10^6$ g-mol min^{-1} g^{-1})		
	CO_2 (alone)	CO_2+H_2	CO_2+N_2
650 °C	67	67	72
750 °C	77	70	71
1150 °C	70	54	71

carbon–carbon dioxide reaction than for the carbon–water vapour reaction, in contrast to normal gasification practice where the carbon–steam reaction is noticeably faster than the carbon–carbon dioxide reaction.

IV. Discussion of Results

Of the reactions studied, that between water vapour and carbon is the only one in which the reaction rate can be correlated with the temperature pretreatment history of the carbon sample. The experimental results shown in Figure 1 indicate that there is some relationship between the rate of reaction and the residual oxygen bound as $\geq C-O-$ in the carbon.

The behaviour of the other dissociated gases indicates that there is no preferential action by atomic hydrogen, atomic oxygen, or dissociated carbon dioxide, that is, CO + atomic oxygen, on the different carbons used in this study. There is some indication that there is a small overall drop in reactivity with increasing temperature of preparation, but this is negligible in comparison with the effect obtained with water vapour. However, when gases in the molecular state are reacted with the carbons, hydrogen shows the characteristic behaviour of the dissociated water vapour (Blackwood 1959) and carbon dioxide reactivity also shows a tendency to follow the change in oxygen content (Blackwood and Ingeme 1960).

Garten and Weiss (1957) have indicated that some of the oxygen present in carbons may be explained by the presence of pyran structures which they have referred to as chromene structures. These structures are a heterocyclic oxygen-containing ring involving an activated $>CH_2$ or $>CHR$ group. Their studies have also suggested that a maximum concentration of such structures may be expected in carbons prepared at 800 °C. On severe pyrolysis of these carbons, such as heating to a temperature of 1250 °C, it is found that the greater proportion of chemically bound oxygen is liberated as oxides of carbon, and there is evidence that groups such as the above appear mostly as carbon monoxide. These groups can act as either oxidizing or reducing centres and may provide the mechanism for electron exchange that is associated with the oxidation or reduction of carbon by oxygen or hydrogen. It is suggested that they may be the groups largely responsible for the change in reactivity of the carbons to dissociated water vapour with change in temperature of char preparation.

The results indicate that the atomic species can react completely at the surface of the carbon and that the reaction rate is not influenced by the nature or number of oxygen-containing groups. Since with molecular species the reaction rate is controlled by the oxygen groups and with atomic species is independent of them, the function of such oxygen groups appears to be the production of atomic reactants.

As pointed out earlier, the products of breakdown of water vapour in the R.F. field are hydrogen atoms and hydroxyl radicals. On reacting these products with carbon, carbon monoxide and hydrogen are formed, and it therefore appears that dissociation of the OH radicals must occur. This must be brought about by the action of the active centres with the production of atomic hydrogen and atomic oxygen, the latter then reacting to form carbon monoxide. This theory is supported by the fact that the rate of gasification is dependent in this case on the number of active centres.

The high temperature–high pressure reaction can now be described in terms of systems in which the reactant gases in the molecular state are chemisorbed at active sites which are electronically activated by suitable oxygen groups. Here electron exchange can take place and the molecules are split into atomic species. These species then react immediately with an adjacent carbon atom to form a molecule of the product gas. Hence the active sites are not preferentially destroyed during gasification, a fact which is confirmed by the constancy of the residual oxygen content (Blackwood 1959). The formation of atomic oxygen has also been suggested as the elementary process in the oxidation of carbon by molecular oxygen (Blackwood and McTaggart 1959).

The mechanism of the retardation of both the carbon–steam and carbon–carbon dioxide reactions may not be as simple as suggested by Gadsby *et al.* (1948) and by Long and Sykes (1948). These authors postulated that, in the case of the carbon–steam reaction, both hydrogen and oxygen could compete equally for active sites and that the retarding action of hydrogen was due to occupation by this gas of sites that could react with oxygen. In this case, carbon monoxide was not a retardant, apart from its effect on the normal reverse reaction. A similar argument was developed for the carbon–carbon dioxide reaction where carbon monoxide was found to be a retardant and it was suggested also that hydrogen would inhibit the latter reaction. It is clear that if hydrogen is to inhibit by a process of occupation of active sites and if it is adsorbed preferentially to carbon monoxide, then it should strongly inhibit the carbon–carbon dioxide reaction. However, experimental evidence (Blackwood and Ingeme 1960) has shown that, provided complications by water gas shift reactions are eliminated, hydrogen has no retarding action on the carbon–carbon dioxide reaction. Further, the present work indicates that hydrogen has no effect on the rate of oxidation of carbon by atomic oxygen, produced by the breakdown of carbon dioxide in the field, also neither atomic hydrogen, molecular hydrogen, nor water vapour inhibits the oxidation of carbon by atomic oxygen. It is therefore concluded that the inhibiting effect is due to reduction in concentration of the atomic species, and that this effect is specific; thus, hydrogen inhibits only the carbon–steam reaction and carbon monoxide only the carbon–carbon dioxide reaction. The action can be explained by a reverse process in which the concentration of atomic species is lowered.

On the above basis the individual steps in the carbon–carbon dioxide reaction can be represented thus:

$$CO_2 \underset{j_1}{\overset{i_1}{\rightleftarrows}} CO + (O), \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$C + (O) \xrightarrow{i_2} CO. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

This process is the same basically as that proposed by Reif (1952) and leads to the rate equation

$$R = \frac{i_1 p_{CO_2}}{1 + j_1/i_2 p_{CO} + i_1/i_2 p_{CO_2}}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

which is identical in form with that derived by Gadsby *et al.* (1948).

Applying the same method to the carbon–steam reaction:

$$H_2O \xrightarrow{i_1} (H)(OH) \underset{j_2}{\overset{i_2}{\rightleftharpoons}} H_2 + (O), \quad \ldots\ldots\ldots\ldots\ldots (4)$$

$$C + (O) \xrightarrow{i_3} CO, \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

which leads to the rate equation

$$R = \frac{i_1 p_{H_2O}}{1 + j_2/i_2 p_{H_2} + (i_1/i_3 + i_1/i_2) p_{H_2O}}. \quad \ldots\ldots\ldots (6)$$

The above treatment is simple and it is not suggested that it necessarily represents the complete mechanism of the reaction, since it has been shown (Blackwood and McGrory 1958) that at high pressures the effect of methane formation must be taken into account. At atmospheric pressure a more rational system is that hydrogen can react with a hydroxyl radical to reform water and atomic hydrogen, thus

$$H_2 + (OH) \rightleftharpoons H_2O + H, \quad \ldots\ldots\ldots\ldots\ldots (7)$$

and that

$$H + H \rightarrow H_2. \quad \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

The authors have determined experimentally that the reverse reaction in equation (7) does take place and the forward reaction is known (Geib 1936). Reaction (8) undoubtedly takes place at a third body such as the carbon surface.

V. Acknowledgments

The authors are indebted to Mr. A. Ingeme for valuable assistance in the analytical and experimental work, to Mr. A. Walsh of the Division of Chemical Physics, C.S.I.R.O., for the spectroscopic determination of OH radicals, and to Dr. H. R. C. Pratt and Mr. R. G. Thomas for their cooperation in making this work possible.

VI. References

Blackwood, J. D. (1959).—*Aust. J. Chem.* **12**: 14.

Blackwood, J. D., and Ingeme, A. T. (1960).—*Aust. J. Chem.* **13**: (in press).

Blackwood, J. D., and McGrory, F. (1958).—*Aust. J. Chem.* **11**: 16.

Blackwood, J. D., and McTaggart, F. K. (1959).—*Aust. J. Chem.* **12**: 114.

Broida, H. P., and Gaydon, A. G. (1953).—*Trans. Faraday Soc.* **49**: 1190.

Broida, H. P., and Kane, W. R. (1953).—*Phys. Rev. Lond.* **89**: 1053.

Gadsby, J., Long, F. J., Sleightholm, P., and Sykes, K. W. (1948).—*Proc. Roy. Soc.* A **193**: 351.

Garten, V. A., and Weiss, D. E. (1957).—*Aust. J. Chem.* **10**: 309.

Geib, K. H. (1936).—*Ergebn. exakt. Naturwiss.* **15**: 44.

Long, F. J., and Sykes, K. W. (1948).—*Proc. Roy. Soc.* A **193**: 377.

Reif, A. D. (1952).—*J. Phys. Chem.* **56**: 785.

Rodebusch, W. H., Wende, C. W. J., and Campbell, R. W. (1937).—*J. Amer. Chem. Soc.* **59**: 1924.

Sanders, T. M., Schawlow, A. L., Dousmanis, G. C., and Townes, C. H (1954).—*J. Chem Phys.* **22**: 245.

Sihvonen, V. (1933).—*Ann. Acad. Sci. Fenn.* A **38** (4): 22.

Tsuchuja, T. (1954).—*J. Chem. Phys.* **22**: 1784.

THE VARIABLE ELECTRONEGATIVITY METHOD

IV. GLYOXALINE, ITS CATION AND ANION

By R. D. Brown* and M. L. Heffernan†

[*Manuscript received April* 9, 1959]

Summary

Results of VESCF calculations of the π-electron distribution and ionization potentials in glyoxaline and its cation and anion are reported. The electron distributions in these systems are in accordance with the electrophilic substitution reactions observed for glyoxaline and bear out the concept of electronegativity reversal in the anion previously suggested to account for chemical properties of glyoxaline.

The dipole moment of glyoxaline, evaluated from the calculated π-electron distribution, agrees with the observed moment, providing further vindication for the VESCF method and support for the suggestion that σ-bond polarizations in heterocycles are slight.

Values of the Hückel coulomb and resonance parameters required to make the Hückel method reproduce the VESCF results for these heterocycles are considered in some detail and it seems feasible to set up empirical rules for selecting appropriate values for the coulomb parameters.

I. Introduction

One of the recent concepts introduced in an attempt to account for the chemistry of heterocyclic compounds in terms of Hückel molecular-orbital calculations is that of electronegativity reversal (Bassett and Brown 1954; Brown 1955; Bassett, Brown, and Penfold 1956). In the present context the electronegativities are judged from the proportions of the total π-electron charge gained by the various conjugated atoms in a simple monocyclic compound.‡ The strongest piece of experimental evidence leading to this hypothesis is the change in the orientation of electrophilic substitution in glyoxaline in alkaline solution, where it has been demonstrated (Brown *et al.* 1953; Ridd 1955) that the substitution takes place in the anion obtained by loss of the proton from the secondary nitrogen; electrophilic substitution in acidic media presumably occurs in the free base or the cation obtained by protonation of the tertiary nitrogen.

* Department of Chemistry, University of Melbourne.

† Department of Chemistry, University of Melbourne; present address: Department of Chemistry, University College, London, W.C.1.

‡ This may not always be a suitable criterion of electronegativity, especially when more than one ring occurs in the molecule. For example, in the case of a non-alternant system such as azulene it may be more appropriate to measure electronegativities by the diagonal elements of the VESCF Hamiltonian matrix and then the least electronegative carbon atom carries the highest π-electron density (Brown and Heffernan 1960).

Attempts to vindicate this concept theoretically have previously been confined to a SCF treatment of an extreme case (Brown and Penfold 1957) and a variable-electronegativity SCF treatment of the pyrrole anion (Brown and Heffernan 1959*b*). The latter showed that electronegativity reversal of tertiary nitrogen relative to carbon occurs if the general level of π-electron densities exceeds a critical value which is in the vicinity of 1·2. It therefore seems useful to apply the VESCF procedure to glyoxaline to attempt to throw more light on the connection between the chemistry of this molecule and the electronegativity reversal concept. The present calculations also were designed to contribute to the problem of devising sets of parameters suitable for Hückel molecular-orbital calculations on heterocycles.

II. Details of Calculations

The details of the VESCF method have been fully set out in previous papers (Brown and Heffernan 1958 ; Brown and Heffernan 1959*a*, 1959*b*). There are no accurate experimental data on the geometry of glyoxaline itself and so, to reduce the labour of computing integrals, the geometry of pyrrole, used in Part II of this series (Brown and Heffernan 1959*a*), was assumed. This meant that most of the penetration integrals obtained for pyrrole and the pyrrole anion (Parts II and III) could be used for the present calculations. The introduction of tertiary nitrogen called for calculation of some penetration integrals involving this atom for internuclear distances differing slightly from those in pyrrole, and these were obtained by short graphical interpolations from values previously calculated accurately. The slight numerical uncertainty thus introduced was considered to be unimportant in view of the lack of precise knowledge of the geometry of the molecule.

Table 1

Assumed geometry for glyoxaline, the cation and anion

	Glyoxaline	Anion	Cation
N_1–C_2	1·38$_3$ A	1·38$_3$ A	1·35$_0$ A
N_1–C_5	1·38$_3$	1·38$_3$	1·37$_0$
C_4–C_5	1·42$_9$	1·42$_9$	1·38$_3$
C_2–C_3	1·37$_1$	1·38$_3$	1·35$_0$
$N_1C_2N_3$	108·1°	109·0°	108·9°
$C_2N_3C_4$	107·5°	109·0°	107·0°
$N_3C_4C_5$	107·5°	107·5°	108·9°
$C_5N_1C_2$	109·0°	109·0°	107·0°

The geometry assumed for glyoxaline is not immediately suitable for the anion because in the latter the two nitrogens are symmetrically equivalent. To minimize the number of new integrals required a geometry closely allied to the geometry used for glyoxaline was adopted. It is summarized in Table 1.

The geometry of the cation (Table 2) in which the two nitrogens are again symmetrically equivalent was adapted from experimental data for histidine in a manner similar to that of Garfinkel and Edsall (1958). Where necessary, values for penetration integrals were obtained by short graphical interpolation of accurately calculated values.

Values of all penetration integrals obtained by interpolation and used in the present work are listed in Table 2. In addition some integrals involving the two nitrogen atoms are required and have no analogue in pyrrole. These were directly calculated from tables, for the appropriate internuclear separation.

TABLE 2

PENETRATION INTEGRALS OBTAINED BY INTERPOLATION

Glyoxaline	(3 : 22)	$1{\cdot}37_1$ A	1·51 eV		(3 : 55)	$2{\cdot}25_8$ A	0·49 eV
	(2 : 33)	$1{\cdot}37_1$	0·71		(5 : 33)	$2{\cdot}25_8$	0·003
	(3 : 44)	$1{\cdot}42_9$	1·42		(1 : 33)	$2{\cdot}22_9$	6·319*
	(4 : 33)	$1{\cdot}42_9$	0·55		(3 : 11)	$2{\cdot}22_9$	0·528*
Glyoxaline anion	(1 : 44)	$2{\cdot}26_7$ A	0·48 eV		(4 : 11)	$2{\cdot}26_7$ A	$0{\cdot}00_2$ eV
	(1 : 33)	$2{\cdot}25_1$	0·516*				
		A		C			
Glyoxaline cation	(1 : 22)	$1{\cdot}35_0$ A	10·115* eV	1·64 eV	(2 : 11)	$1{\cdot}35_0$ A	0·85 eV
	(1 : 33)	$2{\cdot}18_4$	6·448*	0·56	(4 : 11)	$2{\cdot}24_1$	$0{\cdot}00_5$
	(1 : 44)	$2{\cdot}24_1$	6·262*	0·50	(4 : 22)	$2{\cdot}18_7$	0·02
	(1 : 55)	$1{\cdot}37_0$	9·968*	1·51	(4 : 33)	$1{\cdot}37_0$	0·71
					(4 : 55)	$1{\cdot}38_3$	0·68

* Calculated from tables of integrals.

In the cation, penetration integrals involving the nitrogen atom were also calculated directly from tables. These additional integrals are included in Table 2. For all three conjugated systems here studied the core-attraction terms have been treated in the two alternative ways, A and B, described in Part II of this series. In addition a third method, which will be referred to as method C, differing from method A only when there are atoms present in the conjugated system with core charges differing from unity, was tried for the glyoxaline cation. In method C the core-attraction integral $(\mu \mid \mathbf{V}_\nu \mid \mu)$ is written

$$(\mu \mid \mathbf{V}_\nu \mid \mu) = (\mu \mid \mathbf{V}_\nu^\bullet \mid \mu) - X_\nu \gamma_{\mu\nu}, \quad \ldots\ldots\ldots\ (1)$$

where $\mathbf{V}_\nu^\bullet$ is the field of the uncharged atom ν obtained by adding X_ν π-electrons to the $2p\pi$-orbital on ν, X_ν being the core charge of atom ν. The term $(\mu \mid \mathbf{V}_\nu^\bullet \mid \mu)$ in (1) is evaluated theoretically using Slater functions in method C, while in method B it is neglected. The penetration integrals involving the secondary nitrogen atom have values which differ considerably according as method A or C is used for their evaluation. The values are listed in Table 2.

The core resonance integrals for carbon-carbon bonds were, as in previous calculations, evaluated using the Pariser-Parr (1953) formula. The carbon-nitrogen resonance integrals are customarily regarded as adjustable parameters in

calculations of this type and have usually been estimated from ultraviolet spectroscopic intervals. It has been shown previously (Brown and Heffernan 1959*b*) that the charge distribution is insensitive to the value selected for β_{CN} and so in the present work suitable values were estimated from studies on other systems. For glyoxaline the values $\beta_{12}=\beta_{51}=-2\cdot30$ eV (the pyrrole value, Brown and Heffernan 1959*a*) and $\beta_{23}=\beta_{34}=-2\cdot00$ eV (estimated from the pyridine value, Brown and Heffernan (1959*c*) allowing for the greater bond length in glyoxaline) were used. For the carbon-nitrogen bonds in the cation and anion the value $\beta_{CN}=-2\cdot30$ eV was used.

TABLE 3

VALUES OF VESCF QUANTITIES : GLYOXALINE

Quantity*	A†	B‡	Quantity*	A†	B‡
Z_1	4·028	4·017	α_1	—55·984 eV	—53·027 eV
Z_2	3·191	3·240	α_2	—49·747	—46·101
Z_3	3·891	3·838	α_3	—48·875	—46·543
Z_4	3·236	3·238	α_4	—47·039	—43·905
Z_5	3·204	3·218	α_5	—49·333	—45·595
P_1	1·632	1·668	F_{11}	—15·049	—12·412
P_2	1·171	1·029	F_{22}	—10·578	—6·407
P_3	1·025	1·178	F_{33}	—8·859	—6·821
P_4	1·040	1·034	F_{44}	—9·021	—5·651
P_5	1·132	1·092	F_{55}	—10·361	—6·509
I_1	25·822 eV	25·646 eV	F_{12}	—4·122	—4·196
I_2	10·862	11·420	F_{23}	—5·054	—5·044
I_3	14·031	13·283	F_{34}	—4·052	—3·995
I_4	11·380	11·405	F_{45}	—5·589	—5·653
I_5	11·017	11·172	F_{51}	—4·113	—3·999

* The symbols in Tables 3, 4, and 5 have the following meanings :

Z_μ, effective nuclear charge for the atomic orbital χ_μ,

P_μ, π-electron density on atom μ,

I_μ, valence-state ionization potential of atom μ,

α_μ, core resonance integral of atom μ,

$F_{\mu\nu}$, Hartree-Fock Hamiltonian matrix element between atomic orbitals χ_μ and χ_ν.

A full account of these quantities has been given in previous papers of this series, or in references cited therein.

† Method A for treating core attractions.

‡ Method B for treating core attractions.

III. π-Electron Densities

The values obtained for the π-electron distribution in glyoxaline, the anion and the cation, are shown in Tables 3, 4, and 5. For the free base the charges on the three carbon atoms come out to be just greater than unity, the relative values differing when the alternative procedures are used for core attractions. Procedure A yields the highest charge at the 2-position, procedure B the highest charge at the 5-position. For the anion and cation methods A and B also lead to slightly different charge distributions. As we have indicated previously (Brown and Heffernan 1958, 1959*a*) it seems likely that the most appropriate

treatment of core attractions lies somewhere between methods A and B; for simplicity a simple average of the two sets has been taken (Fig. 1).* These values for the π-electron distribution are in agreement with the chemical properties referred to in Section I. Bromination under mildly acidic conditions, in which there is only slight preference for 5-substitution as opposed to 2-substitution (Balaban and Pyman 1922) presumably involves the free base and the lack of

TABLE 4

VALUES OF VESCF QUANTITIES: GLYOXALINE ANION

Quantity*	A	B	Quantity*	A	B
Z_1	3·849	3·814	α_1	—42·557 eV	—40·204 eV
Z_2	3·158	3·197	α_2	—41·044	—37·859
Z_4	3·172	3·188	α_4	—40·387	—37·376
P_1	1·146	1·248	F_{11}	—2·111	—0·061
P_2	1·264	1·149	F_{22}	—2·748	+0·975
P_4	1·222	1·177	F_{44}	—2·356	+0·851
I_1	13·438 eV	12·941 eV	F_{12}	—4·735	—4·777
I_2	10·487	10·935	F_{34}	—4·971	—4·837
I_4	10·653	10·830	F_{45}	—4·047	—4·221

* See first footnote to Table 3.

TABLE 5

VALUES OF VESCF QUANTITIES: GLYOXALINE CATION

Quantity*	A	B	C†	Quantity*	A	B	C†
Z_1	4·088	4·053	4·068	α_1	—62·305	—59·881	—62·233
Z_2	3·219	3·283	3·254	α_2	—58·758	—54·867	—57·855
Z_4	3·253	3·255	3·254	α_4	—55·329	—52·065	—54·769
P_1	1·462	1·562	1·519	F_{11}	—21·463	—19·391	—21·588
P_2	1·090	0·905	0·987	F_{22}	—18·530	—14·048	—17·291
P_4	0·993	0·986	0·987	F_{44}	—16·409	—12·952	—15·742
I_1	26·723 eV	26·198 eV	26·425 eV	F_{12}	—4·732	—4·707	—4·727
I_2	11·182	11·919	11·587	F_{34}	—4·408	—4·166	—4·273
I_4	11·568	11·600	11·594	F_{45}	—5·343	—5·473	—5·417

* See first footnote to Table 3.

† Method C for treating core attractions.

strong orientation may be understood in terms of the π-electron densities for the free base. Nitration, in which the 4- and 5-positions are exclusively attacked (Fargher and Pyman 1919; Balaban and Pyman 1922), has been achieved under strongly acidic conditions and presumably proceeds through the glyoxaline cation. For systems as unreactive as cations there is growing evidence (Bassett and

* Averaging the results amounts to using reduced values of penetration integrals as compared with those obtained by direct numerical evaluation using Slater functions; Pariser and Parr (1953) have suggested that reduced values should be used.

Brown 1954 ; Brown and Heffernan 1956 ; Brown 1958 ; Brown and Harcourt 1959) that the orientation tends to follow localization energies rather than π-electron densities. It does not seem worthwhile at the present stage to use the VESCF method to calculate localization energies for the glyoxaline cation (it is hoped that the labour of such calculations will shortly be considerably reduced by the development of empirical rules for estimating VESCF Hamiltonian matrix elements along the lines explored in Section VI of the present paper) but localization energies derived by the simple Hückel method (Bassett and Brown 1954 ; Brown 1955) indicate a strong orientation in favour of the 4- (or 5-) position in electrophilic substitution.

The observations (Brown *et al.* 1953 ; Ridd 1955) of preferential electrophilic substitution at the 2-position in the anion are in agreement with the π-electron densities for the anion shown in Figure 1. The small difference in charge between the 2- and the 4- (or 5-) position accords with the observation that in diazonium coupling with *p*-bromodiazonium compounds some substitution at the 4-position is also observed (Fargher and Pyman 1919) and that with diazotized aniline the 2,4,5-trisbenzeneazoglyoxaline is easily formed (Fargher and Pyman 1919).

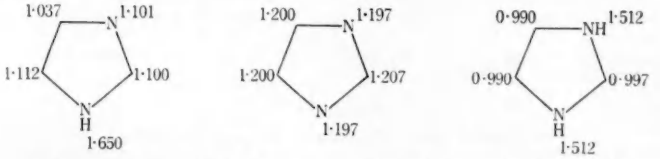


Fig. 1.—π-Electron distributions (average of results by methods A and B).

Since the relative rates of substitution at two different points in a given reactive substrate depend not only on the difference in π-electron densities at those positions but also on the selectivity factor of the electrophil (Brown and Nelson 1953), the electron densities given in Figure 1 cannot be used to predict whether, for example, bromination or diazonium coupling will show a stronger degree of orientation. The bromonium cation is a more vigorous, and hence less discriminating, electrophil than the diazonium cation and so, other things being equal, a less selective orientation might be expected in bromination than in diazonium coupling. For this reason, and also because the observations on bromination of glyoxaline are inadequate for deducing partial rate factors for the several positions of substitution, the data in Figure 1 are in harmony with the currently available observations on electrophilic substitution in glyoxaline. Moreover we would not wish to assert too high a degree of reliability for the averaged π-electron density values. In view of present uncertainties as to the optimum values of penetration integrals it would probably not be pessimistic to consider the data in Figure 1 to be current best estimates with a probable uncertainty of $\pm 0{\cdot}02$ or so in any one value.

Since the data for the anion in Figure 1 show that the nitrogen atoms carry the smallest charge, the present calculations support the concept of electronegativity reversal for this system, as discussed in Section I.

IV. Dipole Moment of Glyoxaline

The π-electron distribution obtained for glyoxaline may be used to estimate the molecular dipole moment. On the assumption that the σ-bonds are unpolarized, so that the core contribution to the dipole moment comes from the nitrogen lone-pair hybridization moment and a small hydrogen orbital hybridization moment (see Part I), the calculated values are (see Table 6) $3 \cdot 7_5$ and $4 \cdot 5_3$ D from methods A and B respectively for core attractions. The experimental

Table 6

DIPOLE MOMENT OF GLYOXALINE

Contribution	Component I* (D)	Component II† (D)
Hybridization on N	1·594‡	0·787
	1·616§	0·798
Hybridization on hydrogens	0·182	0·090
π-Electrons	1·828‡	0·159
	2·606§	0·156

Resultant moment $3 \cdot 7_5$ (D) at angle 16° 4′ with N–H‡
$4 \cdot 5_3$ (D) at angle 13° 20′ with N–H§

* Parallel to N–H. † Perpendicular to N–H.
‡ Method A. § Method B.

moment in solution in a non-polar solvent is about 3·8 D (Wesson 1948). Thus, as was the case for formaldehyde (Brown and Heffernan 1958) and pyrrole (Brown and Heffernan 1959*a*), the observed dipole moment can be fully accounted for without invoking σ-bond polarization, supporting our contention that σ-bond polarization in these heterocyclic systems is small enough to be neglected for most purposes.

The direction of the molecular dipole moment is also predicted in the present calculations (see Table 6). It may ultimately be possible to compare this with the direction determined experimentally by, say, the microwave technique.

V. Ionization Potentials

The VESCF calculations described here yield values for π-electron ionization potentials which are listed in Table 7. In the free base and anion there are other

Table 7

π-ELECTRON IONIZATION POTENTIALS

	Method	First	Second	Third
Glyoxaline ..	A	14·507 eV	15·022 eV	19·369 eV
	B	11·101	12·025	16·259
Anion	A	6·167	6·632	10·317
	B	3·036	4·032	7·492
Cation ..	A	22·061	24·091	27·366
	B	18·540	21·573	24·417
	C	21·357	23·959	26·982

loosely-bound electrons—the *n*-electrons on the tertiary nitrogen atoms—which have comparable energies. The ionization potentials of these, estimated by the method described for formaldehyde (Brown and Heffernan 1958), are 15·0 eV for glyoxaline and 14·0 eV for the anion, these being estimated using the π-electron densities on the tertiary nitrogen atoms given in Figure 1. As indicated in Part III of this series, all the present estimates of ionization potentials are probably too great, but there is little doubt that in the free base the first π-electron ionization potential is slightly less than that of the *n*-electrons, while in the anion the π-electron ionization potentials are much lower than that of the *n*-electrons.

VI. Parameters for the Hückel Method

The most satisfactory justification for the Hückel MO method would seem to be that it is an empirical calculus for roughly reproducing VESCF results; its success depends upon the possibility of devising sufficiently simple rules for estimating VESCF Hamiltonian matrix elements. Now the VESCF Hamiltonian matrix includes elements of appreciable magnitude corresponding, in the Hückel formalism, to resonance integrals between non-neighbour positions. It has been customary to ignore such non-neighbour terms in most Hückel method calculations and the Hückel calculus is sufficiently flexible for it to be possible to reproduce the VESCF π-electron densities by adjusting the coulomb parameters (i.e. values of diagonal matrix elements) to compensate for the neglect of the non-neighbour interaction terms (which was demonstrated for pyrrole in Part II). However, if this " simple " Hückel method is used, the values of orbital energies do not agree with the VESCF values. Alternatively, if the " simple " Hückel method* is used and the coulomb parameters adjusted to reproduce the VESCF orbital energies, then the calculated π-electron densities do not agree with the VESCF values.† It therefore seems preferable to consider the problem of setting up a more complete Hückel method in which all interaction terms are included, that is, of seeking empirical rules for estimating all VESCF Hamiltonian matrix elements. In this section the VESCF results obtained in Parts II, III, and the present paper will be analysed to this end.

In the Hückel method it is expedient to express all matrix elements in " Hückel units ", that is, multiples of β^0, the Hückel resonance integral of a benzene bond. McWeeny and Peacock (1957) have estimated β^0 to be −4·79 eV. The VESCF matrix elements for pyrrole, the pyrrole anion, glyoxaline, its cation and anion, are given in Table 8 in Hückel units.

(i) *Coulomb Parameters.*—For the diagonal matrix elements a fairly systematic pattern emerges. The coulomb terms for cations are all considerably larger than for uncharged systems, and anions have still smaller values for the coulomb terms but no simple rules have been devised for predicting absolute values of these diagonal elements. However, only differences in coulomb terms

* This phrase will be taken to mean the Hückel method neglecting all non-neighbour resonance integrals and taking resonance integrals of all carbon-carbon bonds to be equal.

† This has the important consequence for applications to theories of chemical reactivity that one should not expect a single set of " simple " Hückel coulomb parameters to be suitable for both π-electron density and localization energy calculations.

are significant for determining π-electron densities or localization energies and some simple rules for predicting differences have been discovered. These are based on values of coulomb parameters relative to those of carbon atoms separated from any heteroatom by at least one carbon atom. Thus in pyrrole or the pyrrole anion the coulomb term of the 3-position has been taken as origin. For the glyoxaline anion or cation no atom is sufficiently removed from the heteroatoms to be used as reference atom but the 2-position is subject to auxiliary inductive effects from both heteroatoms, the 4-position from one heteroatom only ; if the coulomb parameters for these two positions are thus taken to be in

TABLE 8

VESCF HAMILTONIAN MATRIX ELEMENTS IN HÜCKEL UNITS*

μν†	Pyrrole	Pyrrole Anion‡	Glyoxaline	Glyoxaline	
				Anion	Cation
11	2·77	0·17	2·87	0·23	4·27
22	1·71	0·16	1·77	0·19	3·41
33	1·46	0·09	1·63	0·23	4·27
44	1·46	0·09	1·53	0·16	3·07
55	1·71	0·16	1·76	0·16	3·07
12	0·84	1·11	0·87	0·99	0·99
23	1·19	1·07	1·05	0·99	0·99
34	0·77	0·87	0·84	1·02	0·90
45	1·19	1·07	1·17	0·86	1·13
51	0·84	1·11	0·85	1·02	0·90
13	—0·17	—0·16	—0·18	—0·14	—0·18
14	—0·17	—0·16	—0·15	—0·14	—0·06
24	—0·04	—0·15	—0·05	—0·16	—0·21
25	—0·19	—0·13	—0·21	—0·16	—0·21
35	—0·04	—0·14	—0·05	—0·14	—0·06

* Mean of values by methods A and B.
† Subscripts of $F_{\mu\nu}$.
‡ For $\beta_{CN} = -2{\cdot}83$ eV.

the proportion 2 : 1 a set of coulomb terms may immediately be obtained. If the auxiliary inductive parameter for the 4-position of glyoxaline is taken to be the same as in the glyoxaline anion this again provides a reference value for the other coulomb parameters. When this type of analysis is applied to the diagonal elements in Table 8 the resultant relative values of coulomb parameters, h, and auxiliary inductive parameters, h', are found to be :

Tertiary Nitrogen	Secondary Nitrogen
$h = 0{\cdot}08,\ 0{\cdot}08,\ 0{\cdot}10$	$h = 1{\cdot}31,\ 1{\cdot}37,\ 1{\cdot}54$
$h' = 0{\cdot}03,\ 0{\cdot}07$	$h' = 0{\cdot}25,\ 0{\cdot}26,\ 0{\cdot}34$

Thus reasonably constant values of the coulomb and auxiliary inductive parameters are found for several different systems even when the overall charge of the system varies by one unit. Furthermore for both secondary nitrogen and

tertiary nitrogen the coulomb parameter exhibits an inverse correlation with the π-electron density of the atom concerned. This is in full harmony with the concept that the electronegativity of the atom decreases as its electron density increases (electronegativity here being measured by the coulomb parameter of the atom).

The present analysis thus appears to enable reasonable estimates of coulomb parameters for the full Hückel method to be made for secondary and tertiary nitrogen. It seems likely that rules for estimating such parameters still more precisely may be forthcoming when the results of VESCF calculations on other heterocyclic systems become available.

(ii) *Resonance Parameters.*—The variation in resonance parameters for nearest neighbours is very much a reflection of the bond lengths assumed in the calculations. This is not surprising since $F_{\mu\nu}=\beta_{\mu\nu}-\frac{1}{2}P_{\mu\nu}\gamma_{\mu\nu}$ ($\mu\neq\nu$), all quantities being widely recognized to depend primarily on bond length. Discrepancies between values of $F_{\mu\nu}$ and bond lengths would be expected only if the assumed geometry were not in accordance with the calculated bond orders. It seems likely that a bond length : VESCF bond order relationship might be established with sufficient reliability to permit an iterative " self-consistent " determination of bond lengths which would circumvent the need to know, or assume, the geometry of the molecule in VESCF calculations.* It is hoped to explore this aspect of the VESCF method in due course.

The values of resonance parameters for next-to-nearest neighbour positions fall into two main groups with values either around $-0\cdot17$ or around $-0\cdot05$. In alternant systems the next-to-nearest neighbour parameters are zero (Coulson and Longuet-Higgins 1947) while in pyridine, which may be regarded as almost alternant (derived from an alternant system by a small perturbation), the values (McWeeny and Peacock 1957 ; Brown and Heffernan 1959*c*) are negligibly small (about $-0\cdot01$). The larger magnitudes occurring in the five-membered ring heterocycles are a reflection of their non-alternant character.† It is hoped to obtain further VESCF data on five-membered ring systems to help in devising rules for prescribing values to be assigned to resonance parameters for next-to-nearest neighbours.

VII. Acknowledgments

The authors are grateful to Imperial Chemical Industries of Australia and New Zealand for generously providing funds for the purchase of a desk calculating machine which was used for much of the computation described above. One of us (M.L.H.) gratefully acknowledges the award of a studentship by the Commonwealth Scientific and Industrial Research Organization.

VIII. References

Balaban, I. E., and Pyman, F. L. (1922).—*J. Chem. Soc.* **121** : 947.
Bassett, I. M., and Brown, R. D. (1954).—*J. Chem. Soc.* **1954** : 2701.
Bassett, I. M., Brown, R. D., and Penfold, A. (1956).—*Chem. & Ind.* **1956** : 892.

* Some principles would still be needed for selecting interbond angles in some cyclic systems.

† For comparison it may be noted that the non-alternant five-membered ring system of the *cyclo*pentadienyl anion has $F_{12}=-0\cdot15\beta^0$.

BROWN, R. D. (1955).—*Aust. J. Chem.* **8**: 100.
BROWN, R. D. (1958).—"Current Trends in Heterocyclic Chemistry." p. 13. (Butterworths Scientific Publications: London.)
BROWN, R. D., DUFFIN, H. C., MAYNARD, J. C., and RIDD, J. H. (1953).—*J. Chem. Soc.* **1953**: 3937.
BROWN, R. D., and HARCOURT, R. D. (1959).—*J. Chem. Soc.* **1959**: (in press).
BROWN, R. D., and HEFFERNAN, M. L. (1956).—*J. Chem. Soc.* **1956**: 4288.
BROWN, R. D., and HEFFERNAN, M. L. (1958).—*Trans. Faraday Soc.* **54**: 757.
BROWN, R. D., and HEFFERNAN, M. L. (1959*a*).—*Aust. J. Chem.* **12**: 319.
BROWN, R. D., and HEFFERNAN, M. L. (1959*b*).—*Aust. J. Chem.* **12**: 330.
BROWN, R. D., and HEFFERNAN, M. L. (1959*c*).—*Aust. J. Chem.* **12**: 554.
BROWN, R. D., and HEFFERNAN, M. L. (1960).—*Aust. J. Chem.* **13**: (in press).
BROWN, H. C., and NELSON, K. L. (1953).—*J. Amer. Chem. Soc.* **75**: 6292.
BROWN, R. D., and PENFOLD, A. (1957).—*Trans. Faraday Soc.* **53**: 397.
COULSON, C. A., and LONGUET-HIGGINS, H. C. (1947).—*Proc. Roy. Soc.* A **192**: 16.
FARGHER, R. G., and PYMAN, F. L. (1919).—*J. Chem. Soc.* **115**: 217.
GARFINKEL, D., and EDSALL, J. T. (1958).—*J. Amer. Chem. Soc.* **80**: 3807.
MCWEENY, R., and PEACOCK, T. E. (1957).—*Proc. Phys. Soc. Lond.* A **70**: 41.
PARISER, R., and PARR, R. G. (1953).—*J. Chem. Phys.* **21**: 767.
RIDD, J. H. (1955).—*J. Chem. Soc.* **1955**: 1238.
WESSON, L. G. (1948).—"Tables of Electric Dipole Moments." (Technology Press: Boston.)

THE VARIABLE ELECTRONEGATIVITY METHOD

V. PYRIDINE, THE PYRIDINIUM CATION, AND THE EVALUATION OF CORE-ATTRACTION INTEGRALS

By R. D. Brown* and M. L. Heffernan†

[*Manuscript received June* 16, 1959]

Summary

A study of pyridine and the pyridinium cation by the VESCF method is reported. Several different methods of evaluating core attractions and electron repulsion integrals have been tried to determine their effect on the resultant energies and orbitals.

Satisfactory results for the π-electron distribution and molecular dipole moment of pyridine are obtained. Values of ionization potentials are little better than those given by other theoretical methods and the variable electronegativity technique does not appear to improve the theoretical treatment of ultraviolet spectra.

Hückel MO parameters, evaluated from the VESCF matrix elements, accord with values found in similar studies of other heterocycles and also with values of parameters found necessary to account for the chemical properties of quinoline.

I. Introduction

In previous papers in this series (Brown and Heffernan 1958, 1959*a*, 1959*b*, 1959*c*) the variable-electronegativity self-consistent field (VESCF) method was applied to various simple heterocyclic systems containing up to five atoms in the conjugated system. The VESCF method has now been applied to two systems containing six conjugated atoms—pyridine and the pyridinium cation. There are several points at which slightly different approximations are used in current SCF calculations and although hitherto we have performed duplicate calculations using two alternative approximations in the VESCF studies it seems desirable to investigate the effects of the alternative approximations on the VESCF results. Pyridine is an appropriate system on which to test the approximations because of the relative wealth of experimental data available for this molecule. In addition to properties depending on the ground-state charge distribution, spectroscopic properties (transition energies and oscillator strengths) have been studied using a CIVESCF procedure.‡

II. Details of Calculations

The main details of the VESCF technique have been described in previous papers (Brown and Heffernan loc. cit.). The various approximations studied in the present work are concerned with the evaluation of the core-attraction

* Department of Chemistry, University of Melbourne.

† Department of Chemistry, University of Melbourne; present address: Department of Chemistry, University College, London, W.C.1.

‡ This is a configuration interaction treatment based on VESCF molecular orbitals. Previous workers have based the CI treatment either on the corresponding hydrocarbon molecular orbitals or else on fixed-Z_μ SCF molecular orbitals.

terms $(\mu \mid \mathbf{V}_\nu \mid \mu)$ which enter in the equation for the core coulomb integral α_μ, and with the evaluation of electron repulsion integrals γ_μ which appear in several places in the calculation. The expression for the core coulomb integral is:

$$\alpha_\mu = -I_\mu + \sum_{\nu \neq \mu} (\mu \mid \mathbf{V}_\nu \mid \mu) - \sum_{\lambda} (\lambda : \mu\mu), \quad \ldots\ldots\ldots (1)$$

where the first summation is over all atoms which participate in the conjugation and the second summation is over all other atoms in the molecule (in the systems studied here these are the hydrogen atoms). In method *A* the term $(\mu \mid \mathbf{V}_\nu \mid \mu)$ representing the potential energy of a π-electron in atomic orbital χ_μ owing to the field of core ν is written:

$$(\mu \mid \mathbf{V}_\nu \mid \mu) = (\mu \mid \mathbf{V}_\nu^+ \mid \mu) - \gamma_{\mu\nu}, \quad \ldots\ldots\ldots\ldots (2)$$

where $\mathbf{V}_\nu^+$ is the field of core ν plus a π-electron in χ_ν. For the most common case of cores of unit positive charge $\mathbf{V}_\nu^+$ represents the field of an uncharged atom. In method *A*, $(\mu \mid \mathbf{V}_\nu^+ \mid \mu)$ is calculated theoretically using Slater functions.

In method *B* the core-attraction term is written

$$(\mu \mid \mathbf{V}_\nu \mid \mu) = (\mu \mid \mathbf{V}_\nu^* \mid \mu) - X_\nu \gamma_{\mu\nu}, \quad \ldots\ldots\ldots\ldots (3)$$

where $\mathbf{V}_\nu^*$ is the field of the uncharged atom ν obtained by putting X_ν electrons in the $2p\pi$-orbital χ_ν, X_ν being the core charge with respect to π-electrons, that is,

$$X_\nu = v_\nu - b_\nu - p_\nu, \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where v_ν is the number of valence electrons in atom ν, b_ν the number of σ-bonds in which atom ν participates, and p_ν the number of lone-pair electrons on ν. In method *B*, the neutral atom potential is neglected, so that

$$(\mu \mid \mathbf{V}_\nu \mid \mu) = -X_\nu \gamma_{\mu\nu} \quad \text{(method } B\text{)}. \quad \ldots\ldots\ldots (5)$$

In method *C*, equation (3) is used to represent the core-attraction term, but $(\mu \mid \mathbf{V}_\nu^* \mid \mu)$ is calculated theoretically using Slater functions. In the case of a core of unit-positive charge methods *A* and *C* are identical, of course, so that for pyridine there is no distinction between methods *A* and *C*, although for pyridinium the two procedures yield different results.

There are several procedures for selecting values of the electron repulsion integrals $\gamma_{\mu\nu}$ arising in equations (2), (3), and (5). They are:

Method *T*: the integrals are evaluated theoretically using Slater functions.

Method *P*: the "atoms in molecules" formula of Pariser (1953) is used.

Method *R*: the reciprocal distance approximation, introduced by Pople (1953), is used.

Method *J*: the formula of Mataga and Nishimoto (1957) is used for $\gamma_{\mu\nu}$, that is, in atomic units, $\gamma_{\mu\nu} = 1/(a_{\mu\nu} + r_{\mu\nu})$, the value of the constant $a_{\mu\nu}$ being fixed by the average monocentric value $\frac{1}{2}(\gamma_\mu + \gamma_\nu)$.

Electron repulsion integrals occur elsewhere in the Hamiltonian matrix elements:

$$F_{\mu\mu} = \alpha_\mu + \tfrac{1}{2} P_\mu \gamma_\mu + \sum_{\nu \neq \mu} P_\nu \gamma_{\mu\nu}, \quad \ldots\ldots\ldots\ldots (6)$$

$$F_{\mu\nu} = \beta_{\mu\nu} - \tfrac{1}{2} P_{\mu\nu} \gamma_{\mu\nu}, \quad \ldots\ldots\ldots\ldots\ldots (7)$$

P_μ being the π-electron density on atom μ, $P_{\mu\nu}$ the generalized bond order between atoms μ and ν, and $\beta_{\mu\nu}$ the core-Hamiltonian resonance integral between atoms μ and ν. The two-centre electron repulsion integrals in (**6**) and (**7**) were given the same values (*T*, *P*, *R*, or *J*) as were used in calculating core attractions in each particular calculation but the monocentric repulsion integral in (**6**) was always evaluated from the Paoloni formula (see Brown and Heffernan 1959*a*).

The calculations were based on the precise data for the geometry of pyridine used in our previous calculations on this molecule (Brown and Heffernan 1957)

TABLE 1

VESCF QUANTITIES FOR PYRIDINE

Quantity	*AP*	*BP*	*AT*	*BR*	*BJ*
Z_1	3·890	3·864	3·888	3·851	3·866
Z_2	3·246	3·267	3·250	3·281	3·263
Z_3	3·256	3·247	3·253	3·235	3·251
Z_4	3·257	3·257	3·257	3·267	3·256
I_1	14·018 eV	13·646	13·996 eV	13·467 eV	13·680 eV
I_2	11·496	11·733	11·534	11·897	11·693
I_3	11·604	11·513	11·571	11·367	11·545
I_4	11·616	11·622	11·624	11·734	11·612
α_1	—47·732	—45·456	—51·351	—52·031	—36·412
α_2	—46·331	—43·463	—50·298	—50·892	—34·050
α_3	—45·286	—42·553	—48·961	—49·371	—33·445
α_4	—44·952	—42·432	—48·671	—49·500	—33·392
F_{11}	—9·431	—7·413	—9·449	—7·883	—7·200
F_{22}	—9·212	—6·104	—9·187	—5·659	—6·214
F_{33}	—8·905	—6·275	—8·923	—6·616	—6·253
F_{44}	—8·858	—6·295	—8·858	—6·085	—6·310
F_{12}	—5·106	—5·090	—5·648	—6·024	—4·336
F_{23}	—4·870	—4·881	—5·410	—5·831	—4·143
F_{34}	—4·737	—4·731	—5·268	—5·681	—3·999
F_{14}	0·803	0·806	0·813	0·830	0·587
F_{25}	0·848	0·842	0·867	0·899	0·598

and the geometry of the pyridinium cation was assumed to be the same as for pyridine (this meant that many of the integrals evaluated for pyridine could be used again in the pyridinium calculations).

The carbon-carbon core resonance integrals were derived from the Pariser-Parr (1953*a*) formula as in our previous calculations. The value of β_{CN} for pyridine, −2·470 eV, was derived by fitting the calculated transition energy of the longest wavelength ultraviolet spectral transition to the experimental value; the calculation was a configuration-interaction treatment of the Pariser-Parr (1953*a*) type, based on benzene orbitals, but using the accurate pyridine geometry. For the pyridinium cation two alternative values of β_{CN} were tried, the pyrrole value (Brown and Heffernan 1959*a*), −2·30 eV, and a value of −2·50 eV which gave better agreement with the first spectral transition of pyridinium (see below).

The values used for penetration integrals and coulomb repulsion integrals have been published previously (Brown and Heffernan 1957). Final values of quantities whose dependence upon effective nuclear charge was included in the computations are given in Tables 1 and 2.

TABLE 2

VESCF QUANTITIES FOR PYRIDINIUM

Quantity	*BP*	*BP*	*AP*	*CP*
β_{CN}	−2·30 eV	−2·50 eV	−2·50 eV	−2·50 eV
Z_1	4·062	4·068	4·117	4·089
Z_2	3·288	3·286	3·231	3·268
Z_3	3·275	3·275	3·303	3·282
Z_4	3·312	3·310	3·315	3·311
I_1	26·321 eV	26·415 eV	27·114 eV	26·738 eV
I_2	11·975	11·951	11·324	11·749
I_3	11·830	11·829	12·149	11·903
I_4	12·250	12·228	12·282	12·239
α_1	−58·131	−58·225	−60·868	−60·452
α_2	−51·605	−51·581	−55·681	−54·484
α_3	−48·430	−48·429	−51·556	−51·145
α_4	−47·950	−47·928	−50·575	−50·465
F_{11}	−19·417	−19·490	−21·424	−21·499
F_{22}	−13·306	−13·325	−17·947	−16·421
F_{33}	−12·471	−12·451	−15·324	−15·087
F_{44}	−12·295	−12·264	−15·124	−14·831
F_{12}	−4·477	−4·711	−4·873	−4·798
F_{23}	−5·028	−5·012	−4·944	−4·990
F_{34}	−4·614	−4·625	−4·605	−4·626
F_{13}	0·231	0·229	0·293	0·244
F_{24}	−0·533	−0·513	−0·455	−0·475
F_{35}	−0·223	−0·231	−0·471	−0·303
F_{14}	0·727	0·725	0·815	0·759
F_{25}	0·712	0·723	0·664	0·717
F_{26}	0·759	0·749	0·779	0·732

The positions of the lower excited states and transition probabilities between states were calculated using configuration-interaction treatments based on the ground state VESCF orbitals. The CI matrix elements have the form (Mataga and Nishimoto 1957 ; McWeeny and Peacock 1957).

$$({}^1\psi_{i\to k} \mid \mathbf{H} \mid {}^1\psi_{i\to k}) = \varepsilon_k - \varepsilon_i + 2(ik \mid ik) - (ii \mid kk), \quad \ldots\ldots \quad (8)$$

$$({}^3\psi_{i\to k} \mid \mathbf{H} \mid {}^3\psi_{i\to k}) = \varepsilon_k - \varepsilon_i - (ii \mid kk), \quad \ldots\ldots\ldots\ldots\ldots \quad (9)$$

$$({}^1\psi_{i\to k} \mid \mathbf{H} \mid {}^1\psi_{j\to m}) = 2(ik \mid jm) - (ij \mid km), \quad \ldots\ldots\ldots\ldots \quad (10)$$

$$({}^3\psi_{i\to k} \mid \mathbf{H} \mid {}^3\psi_{j\to m}) = -(ij \mid km). \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

The notation ${}^3\psi_{i\to k}$ representing, for example, a configuration function describing the triplet configuration derived from the ground-state configuration by transferring an electron from the ith to the kth molecular orbital. The VESCF energy of the ith molecular orbital is ε_i and

$$(ij \mid km) = \int\int \varphi_i(1)\varphi_j(1)\frac{1}{r_{12}}\varphi_k(2)\varphi_m(2)\mathrm{d}\tau_{12}, \quad \ldots\ldots \quad (12)$$

φ_i being the ith molecular orbital. The forms of the φ_i and values of the ε_i used in the present calculations are given in Table 3, based on orbitals and

TABLE 3

VESCF ORBITALS AND ENERGIES FOR PYRIDINE

		ε_i	χ_3	χ_2	χ_1	χ_6	χ_5	χ_4
Method	φ_0 b_2	—18·116 eV	0·3653	0·4398	0·4832	0·4398	0·3653	0·3356
AP	φ_1 b_2	—14·813	0·3436	—0·2288	—0·5299	—0·2288	0·3436	0·6150
	φ_2 a_2	—14·789	0·4902	0·5096	0	—0·5096	—0·4902	0
	φ_3 b_2	—3·351	0·3003	0·2806	—0·5849	0·2806	0·3003	—0·6000
	φ_4 a_2	—3·345	0·5096	—0·4902	0	0·4902	—0·5096	0
Method	φ_0 b_2	—15·455 eV	0·3582	0·4317	0·5076	0·4317	0·3582	0·3360
BP	φ_1 b_2	—12·278	0·3471	—0·2074	—0·5437	—0·2074	0·3471	0·6143
	φ_2 a_2	—11·972	0·5032	0·4968	0	—0·4968	—0·5032	0
	φ_3 b_2	—0·807	0·2972	0·2995	—0·5299	0·2995	0·2972	—0·6026
	φ_4 a_2	—0·526	0·4968	—0·5032	0	0·5032	—0·4968	0
Method	φ_0 b_2	—14·197 eV	0·3593	0·4327	0·5061	0·4327	0·3593	0·3332
BJ	φ_1 b_2	—11·268	0·3463	—0·2096	—0·5396	—0·2096	0·3463	0·6174
	φ_2 a_2	—11·015	0·4990	0·5009	0	—0·5009	—0·4990	0
	φ_3 b_2	—1·758	0·2980	0·2965	—0·5349	0·2965	0·2980	—0·6004
	φ_4 a_2	—1·532	0·5009	—0·4990	0	0·4990	—0·5009	0

TABLE 4

VESCF ORBITALS AND ENERGIES FOR PYRIDINIUM

		ε_i	χ_3	χ_2	χ_1	χ_6	χ_5	χ_4
Method	φ_0 b_2	—26·539 eV	0·2036	0·4148	0·7426	0·4148	0·2036	0·1467
CP	φ_1 b_2	—22·235	0·4620	0·0403	—0·4222	0·0403	0·4620	0·6256
(β_{CN}=	φ_2 a_2	—21·804	0·4469	0·5478	0	—0·5478	—0·4469	0
—2·50 eV)	φ_3 b_2	—11·3522	+0·1242	+0·4626	—0·4736	+0·4626	+0·1242	—0·5629
	φ_4 a_2	—10·139	0·5479	—0·4469	0	0·4469	—0·5479	0
Method	φ_0 b_2	—23·724 eV	0·1995	0·3920	0·7686	0·3920	0·1995	0·1488
BP	φ_1 b_2	—19·712	0·4612	0·0605	—0·4219	0·0605	0·4612	0·6238
(β_{CN}=	φ_2 a_2	—18·968	0·4593	0·5376	0	—0·5376	—0·4593	0
—2·30 eV)	φ_3 b_2	—8·881	0·1204	0·4771	—0·4394	0·4771	0·1204	—0·5679
	φ_4 a_2	—7·344	0·5376	—0·4593	0	0·4593	—0·5376	0
Method	φ_0 b_2	—27·149 eV	0·2062	0·4416	0·7102	0·4416	0·2062	0·1433
AP	φ_1 b_2	—22·557	0·4613	0·0297	—0·4304	0·0297	0·4613	0·6224
(β_{CN}=	φ_2 a_2	—22·721	0·4103	0·5758	0	—0·5758	—0·4103	0
—2·50 eV)	φ_3 b_2	—12·003	+0·0902	+0·4620	—0·5187	+0·4620	+0·0902	—0·5365
	φ_4 a_2	—10·856	0·5758	—0·4103	0	0·4103	—0·5758	0

energies obtained by methods *AP*, *BP*, and *BJ* for pyridine, and Table 4, based on orbitals and energies obtained by methods *AP*, *BP*, and *CP* for pyridinium. The corresponding CI matrix elements are given in Tables 5 and 6.

Oscillator strengths for the various singlet-singlet transitions were calculated from the formula (Mataga and Nishimoto 1957 ; Mulliken and Rieke 1941)

$$f = 1{\cdot}085 \times 10^{11} \omega_{0j} \sum_{r=x,y,z} (M_{0j}^{r})^2, \quad \ldots\ldots\ldots \quad (13)$$

where ω_{0j} is the frequency of the $0 \rightarrow j$ transition and M_{0j}^{r} is the rth component of the transition moment for that transition.

TABLE 5

CONFIGURATION-INTERACTION MATRIX ELEMENTS : PYRIDINE*

Element	*AP*	*BP*	*BJ*
$(^1\psi_{2\rightarrow3} \mid \mathbf{H} \mid {}^1\psi_{2\rightarrow3})$	5·899 eV	5·684 eV	5·644 eV
$(^1\psi_{1\rightarrow4} \mid \mathbf{H} \mid {}^1\psi_{1\rightarrow4})$	5·933	6·146	6·050
$(^1\psi_{2\rightarrow3} \mid \mathbf{H} \mid {}^1\psi_{1\rightarrow4})$	1·025	1·024	0·980
$(^1\psi_{2\rightarrow4} \mid \mathbf{H} \mid {}^1\psi_{1\rightarrow4})$	6·134	6·141	6·513
$(^1\psi_{1\rightarrow3} \mid \mathbf{H} \mid {}^1\psi_{1\rightarrow3})$	6·300	6·291	6·618
$(^1\psi_{2\rightarrow4} \mid \mathbf{H} \mid {}^1\psi_{1\rightarrow3})$	—0·990	—0·985	—0·457
$(^3\psi_{2\rightarrow3} \mid \mathbf{H} \mid {}^3\psi_{2\rightarrow3})$	4·640 eV	4·350 eV	—
$(^3\psi_{1\rightarrow4} \mid \mathbf{H} \mid {}^3\psi_{1\rightarrow4})$	4·668	5·038	—
$(^3\psi_{2\rightarrow3} \mid \mathbf{H} \mid {}^3\psi_{1\rightarrow4})$	—0·229	—0·222	—
$(^3\psi_{2\rightarrow4} \mid \mathbf{H} \mid {}^3\psi_{2\rightarrow4})$	4·225	4·220	3·610
$(^3\psi_{1\rightarrow3} \mid \mathbf{H} \mid {}^3\psi_{1\rightarrow3})$	4·082	4·094	3·475
$(^3\psi_{2\rightarrow4} \mid \mathbf{H} \mid {}^3\psi_{1\rightarrow3})$	—0·229	—0·222	—0·424

* Values for all diagonal matrix elements are relative to the energy of the ground configuration.

TABLE 6

CONFIGURATION-INTERACTION MATRIX ELEMENTS : PYRIDINIUM

Elements	*AP* ($\beta_{CN} = -2{\cdot}50$eV)	*BP* ($\beta_{CN} = -2{\cdot}30$eV)	*CP* ($\beta_{CN} = -2{\cdot}50$eV)
$^1B_1$ type			
$(^1\psi_{2\rightarrow3} \mid \mathbf{H} \mid {}^1\psi_{2\rightarrow3})$	5·289 eV	4·782 eV	5·100 eV
$(^1\psi_{1\rightarrow4} \mid \mathbf{H} \mid {}^1\psi_{1\rightarrow4})$	6·133	6·625	6·397
$(^1\psi_{2\rightarrow3} \mid \mathbf{H} \mid {}^1\psi_{1\rightarrow4})$	1·418	0·885	0·911
$^1A_1$ type			
$(^1\psi_{2\rightarrow4} \mid \mathbf{H} \mid {}^1\psi_{2\rightarrow4})$	6·468	6·317	6·337
$(^1\psi_{1\rightarrow3} \mid \mathbf{H} \mid {}^1\psi_{1\rightarrow3})$	5·619	5·745	5·826
$(^1\psi_{2\rightarrow4} \mid \mathbf{H} \mid {}^1\psi_{1\rightarrow3})$	—0·699	—0·875	—0·894

III. π-Electron Densities

The π-electron densities of pyridine found in the present calculations are summarized in Table 7. As has been pointed out previously (Brown and Heffernan 1957), there are complications which may, so far as is known at present, preclude a direct correlation of chemical properties of pyridine with the π-electron distribution. However, proton magnetic resonance observations (Bernstein

and Schneider 1956) provide relatively direct evidence, especially if the polarization of σ-bonds is negligible (Brown and Heffernan 1958, 1959*a*, 1959*c*), that the π-electron densities fall in the order $3>4>2$. On this basis the results by method *B* are more satisfactory than by method *A*. If, as has been suggested (Brown and Heffernan 1958, 1959*a*, 1959*b*, 1959*c*), the most suitable treatment lies somewhere between these two methods, then it probably lies nearer to method *B* than to *A*. The simple average of methods *AP* and *BP* used in Part IV

TABLE 7

VESCF π-ELECTRON DENSITIES FOR PYRIDINE

Method	P_1	P_2	P_3	P_4
AP	1·029	1·011	0·984	0·982
BP	1·107	0·952	1·004	0·981
AT	1·029	1·006	0·988	0·982
BR	1·150	0·900	1·054	0·938
BJ	1·095	0·964	0·996	0·984

gives a charge distribution in pyridine in which the 2- and 4-positions have the same charge. The VESCF method has not yet been tested sufficiently to tell whether better agreement than this is to be expected of this procedure for calculating charge distributions in heterocyclic systems absolutely.

The treatments *P* and *T* for electron repulsion integrals give virtually the same π-electron distribution, but the change from *P* to method *R* has a larger effect on π-electron densities. This may be attributed to the fact that method *R* gives a particularly large value to $\gamma_{\mu\nu}$ for nearest neighbours—larger than the

TABLE 8

VESCF π-ELECTRON DENSITIES FOR PYRIDINIUM

Method	β_{CN}	P_1	P_2	P_3	P_4
BP	−2·30 eV	1·538	0·893	0·927	0·823
BP	−2·50	1·520	0·899	0·927	0·829
AP	−2·50	1·379	1·055	0·847	0·816
CP	−2·50	1·459	0·948	0·909	0·827

empirical (Paoloni) value for the monocentric repulsion integral. Although method *T* gives a somewhat larger value than method *P* for this integral, it is still well below the Paoloni monocentric value.

The charge distributions obtained by methods *BJ* and *BP* are not greatly different and, furthermore, the charge distribution obtained by Mataga and Nishimoto (1957), using method *BJ* without the variable electronegativity principle, is not very different from that obtained here by method *BP*. There is a similar resemblance between the present VESCF results using the *AP* method and corresponding results (Brown and Heffernan 1959*c*) obtained by a SCF

method in which the variable electronegativity principle was not used. This emphasizes that the major factor affecting the calculated charge distribution is the inclusion or neglect of the coulomb penetration integrals in the core-attraction terms.

The calculated π-electron densities for the pyridinium cation are shown in Table 8. There does not appear to be any experimental data available for this ion which might indicate its charge distribution. The two sets of results for method *BP* using different values of β_{CN} show that the π-electron distribution is insensitive to this resonance integral, perhaps even more so than in the case of pyrrole (Brown and Heffernan 1959*a*), showing that when we are concerned only with the charge distribution, errors arising from uncertainties in values of core resonance integrals may generally be disregarded.

IV. Dipole Moment of Pyridine

The dipole moment of pyridine has been calculated using the VESCF charge distributions and assuming no polarization of σ-bonds. The results are shown in Table 9. The different treatments of core attractions and of electron repulsion

Table 9

Dipole moment of pyridine

Method	π-Moment	N Hybridization	H Hybridization	Total (D)
AP	0·486	1·831	0·188	$2·5_1$
BP	0·520	1·844	0·188	$2·5_5$
AT	0·468	1·830	0·188	$2·4_9$
BR	0·469	1·851	0·188	$2·5_1$
BJ	0·554	1·842	0·188	$2·5_8$
Experiment				2·26,* 2·15†

* Bak, Hansen-Lyngaard, and Rastrup-Andersen (1958).
† DeMore, Wilcox, and Goldstein (1954).

integrals all lead to substantially the same value, which agrees with the experimental value about as closely as in previous calculations (Brown and Heffernan 1958, 1959*a*, 1959*c*). In the present case the nitrogen hybridization moment makes the major contribution to the moment and it is likely that some of the error in the theoretical estimate of the total comes from evaluating the hybridization moment using Slater orbitals.

V. Ionization Potentials

The π-electron ionization potentials of pyridine, calculated by the different VESCF methods, are listed in Table 10. The ionization potential of the nitrogen *n*-electrons, derived as was described in the case of formaldehyde (Brown and Heffernan 1958) and the pyrrole anion (Brown and Heffernan 1959*b*), is also listed in Table 10. Since these estimates of ionization potentials include a systematic error (Brown and Heffernan 1959*b*), it is preferable to compare them

with the value calculated for some suitable reference molecule using the same procedure. Corresponding values of the first ionization potential of benzene are accordingly included in Table 10.

The most recent experimental value for pyridine is 9·23 eV (Watanabe 1957), which is very close to that of benzene, namely, 9·25 eV (Watanabe 1957). The values estimated by methods *AP* and *AT* are nearer to the experimental value than those estimated by methods *BP*, *BR*, or *BJ* ; all methods predict that

TABLE 10

IONIZATION POTENTIALS FOR PYRIDINE (eV)

Method	n	1st π	2nd π	3rd π	Benzene	Pyridine*
AP	$16{\cdot}0_0$	14·79	14·81	18·12	14·64	9·40
BP	$15{\cdot}0_5$	11·97	12·28	15·45	12·66	8·56
AT	$16{\cdot}0_0$	15·34	15·37	19·17	15·26	9·33
BR	$14{\cdot}3_5$	12·96	13·36	17·31	13·76	8·45
BJ	$15{\cdot}1_5$	11·01	11·27	14·20	11·52	8·74

* First ionization potential, estimated using theoretical difference from benzene value and the observed value of 9·25 eV for benzene.

the first π-electron ionization potential lies below the n-electron ionization potential. This may be compared with a recent suggestion, based on rather uncertain and indirect experimental evidence, that the observed ionization potential of pyridine corresponds to n-electron rather than π-electron ionization (Higasi, Omura, and Baba 1956).

The calculated ionization potentials of the pyridinium cation (Table 11) are all considerably higher than those of pyridine, as would be expected for a positively charged entity. No experimental values are available in this case.

TABLE 11

IONIZATION POTENTIALS OF PYRIDINIUM CATION

Method	β_{CN} (eV)	1st π	2nd π	3rd π
AP	—2·50	22·56	22·72	27·15
BP	—2·50	18·96	19·69	24·05
BP	—2·30	18·97	19·72	23·72
CP	—2·50	21·80	22·24	26·56

VI. HÜCKEL PARAMETERS

The VESCF Hamiltonian matrix elements for pyridine and pyridinium (average of values by methods *AP* and *BP*), divided by $\beta^0 = -4{\cdot}79$ eV, give the Hückel parameters (McWeeny and Peacock 1957 ; Brown and Heffernan 1959*c*) shown in Tables 12 and 13. The value of the coulomb parameter for tertiary nitrogen is in harmony with the values found in related heterocycles (Brown and

Heffernan 1959*c*), as is the very small auxiliary inductive parameter at the 2-position of pyridine. The coulomb parameter for the pyridinium nitrogen and its auxiliary inductive parameter are respectively smaller and larger than might have been expected from the values previously found (Brown and Heffernan 1959*c*) for secondary nitrogens in five-membered rings. We hope to study this aspect more fully in due course with calculations on other six-membered ring nitrogen heterocycles.

TABLE 12

HÜCKEL PARAMETERS FOR PYRIDINE (β^0)

Coulomb Parameter	Value	Resonance Parameter	Value
F_{11}	0·176	F_{12}	1·06
F_{22}	0·017	F_{23}	1·02
F_{33}	0·003	F_{34}	0·99
F_{44}	0*	F_{14}	−0·168
		F_{25}	−0·176

* Arbitrarily taken as origin ($F_{44}=1\cdot582\ \beta^0$).

TABLE 13

HÜCKEL PARAMETERS FOR PYRIDINIUM (β^0)

Parameter	Value	Parameter	Value
F_{11}	1·41	F_{13}	−0·054
F_{22}	0·41	F_{24}	0·101
F_{33}	0·04	F_{26}	−0·160
F_{44}	0*	F_{35}	0·073
F_{12}	1·00	F_{14}	−0·161
F_{23}	1·04	F_{25}	−0·145
F_{34}	0·96		

* Arbitrarily taken as origin ($F_{44}=2\cdot86\ \beta^0$).

The resonance parameters for nearest neighbours are all close to the benzene value, as would be expected from the bond lengths in the case of carbon-carbon bonds. In addition the values for the CN bonds support the assumption, often made in Hückel MO calculations, that $\beta^0_{CN}=\beta^0$.

The values of resonance parameters for more distant interactions are interesting. For pyridine the next-to-nearest neighbour interactions are all negligibly small and so were not included in Table 12. However, the 1,4 and 2,5 parameters have values very close to −0·17. Identical results were found by McWeeny and Peacock (1957) in a different, simpler SCF treatment of pyridine. In the case of pyridinium the next-to-nearest neighbour interactions are no longer negligible, in keeping with the greater divergence from alternant character for this system (Brown and Heffernan 1959*c*). In the five-membered ring systems these parameters had values (Brown and Heffernan 1959*c*) either around

$-0\cdot17$ or around $-0\cdot05$ but in pyridinium values of either sign arise. The "para" resonance parameters for pyridinium are also close to the value $-0\cdot17$, suggesting that this value is probably satisfactory for all "para" resonance parameters in six-membered nitrogen heterocycles.

If the "simple" Hückel method, in which all non-neighbour resonance integrals are neglected and all carbon-carbon resonance integrals are given the standard value β^0, is applied to pyridine then for $\beta^0_{CN}=\beta^0$ the values of the nitrogen coulomb parameter and auxiliary inductive parameter needed to reproduce the VESCF charge distribution (average of those for methods *AP* and *BP*) are $h=0\cdot19_5$, $h'=0\cdot03_1$. In a recent study of the chemistry of quinoline using the "simple" Hückel approximation (Brown and Harcourt 1959) the value of h for tertiary nitrogen was assumed to be $0\cdot5$ and then it was found that the value of h' needed to account for the chemistry was around $0\cdot08_5$. Since the theoretical quantities used in the analysis of the chemistry of quinoline depend linearly on h and h' over short ranges of these parameters, the parameter values $h=0\cdot5\times(0\cdot19_5/0\cdot5)=0\cdot19_5$ and $h'=0\cdot08_5\times(0\cdot19_5/0\cdot5)=0\cdot03_2$ would equally account for the observed chemistry of quinoline. Thus the present VESCF results for pyridine are fully compatible with the investigations of quinoline by the simple Hückel method.

Only two coulomb parameters are required to make the simple Hückel method reproduce to better than three decimals the four different VESCF π-electron densities in pyridine, showing that in the Hückel method it is justifiable to neglect auxiliary inductive effects beyond the nearest neighbours of heteroatoms.

VII. Ultraviolet Spectrum of Pyridine

The results of the CIVESCF calculations of the spectrum of pyridine are shown in Table 14. The agreement between predicted and observed transition energies is about as good as has previously been found in Pariser-Parr type treatments (Pariser and Parr 1953*a*; Mataga and Nishimoto 1957; McWeeny and Peacock 1957). The predicted energy of the second N→V transition is too low by about 1 eV, a circumstance which has been observed in other calculations on heterobenzenes (McWeeny and Peacock 1957) and other heterocycles (Brown and Heffernan 1959*a*) and is present in Parr and Pariser's (1953*b*) original calculation on benzene (the effect is minimized in McWeeny and Peacock's (1957) calculations by adjustment of empirical parameters to give the best overall agreement). A detailed study of benzene (Pariser and Parr 1953*b*) has shown that better agreement with experiment is obtained by using a smaller value of γ_{12} than that normally employed. Mataga and Nishimoto (1957) have used a still smaller value of $\gamma_{\mu\nu}$ for nearest neighbours in heterocycle calculations and they obtained noticeably better agreement with experiment for the second π-π^* transition, this improvement extending also to larger nitrogen heterocycles (Mataga 1958). In addition, they used a somewhat different value for β_{CN} ($-2\cdot576$ eV). To determine whether a change in the nearest-neighbour $\gamma_{\mu\nu}$ values or in the value of β_{CN} in the present CIVESCF calculations would improve the agreement with experiment, the functional dependence of the matrix elements

in a Pariser-Parr treatment* of pyridine was determined (Table 15). Then, by assuming that the CIVESCF matrix elements have the same dependence upon β_{CN} and the $\gamma_{\mu\nu}$† the CIVESCF matrix for method *A* was approximately estimated (i) reducing all $\gamma_{\mu\nu}$ for nearest neighbours by 0·4 eV compared with the values previously used, (ii) reducing all $\gamma_{\mu\nu}$ for nearest neighbours 0·4 eV and changing β_{CN} to −2·77 eV. The resultant calculated spectroscopic intervals are listed in Table 16. The change in the $\gamma_{\mu\nu}$ tends to improve the predicted energy of the second N→V transition at the expense of other predicted transition energies.

TABLE 14

π-π^* SPECTRAL TRANSITIONS IN PYRIDINE

Transition Energies (eV)	Excited State	*AP*	*BP*	*BJ*	*MP**	*MN*†	*PP*‡	Observed
(*a*) Singlet-singlet	$^1B_1$	4·89§	4·86§	4·85§	5·0	5·01	4·90	4·90
	$^1A_1$	5·22	5·23	6·11	5·8	6·25	—	6·38
	$^1B_1$	6·94	6·97	6·85	7·2	7·16	—	7·07
	$^1A_1$	7·21	7·20	7·03	7·2	7·23	—	
(*b*) Singlet-triplet	$^3A_1$	3·91	3·93	3·11	3·8	3·28	4·08	3·67
	$^3B_1$	4·43	4·28	—	—	3·92	—	—
	$^3A_1$	4·39	4·39	3·97	—	4·15	—	—
	$^3B_1$	4·88	5·10	—	—	5·24	—	—
Oscillator strengths	$^1B_1$	*c.* 10^{-4}	0·01	0·013	—	0·024	0·05	0·04
	$^1A_1$	5×10^{-4}	4×10^{-4}	0·001	—	0·026	—	0·10
	$^1B_1$	1·026	1·013	1·001	—	1·144	—	1·30
	$^1A_1$	1·169	1·169	1·171	—	1·244	—	

* McWeeny and Peacock (1957).
† Mataga and Nishimoto (1957).
‡ Pariser and Parr (1953*a*).
§ Used for selecting the value of β_{CN}.

The change in both the $\gamma_{\mu\nu}$ and β_{CN} further improves the predicted energy of the second transition without materially altering the agreement between other predicted and observed transition energies.

This overall correspondence between theory and experiment is similar to that found by McWeeny and Peacock (1957) by a similar empirical adjustment of the parameters for pyridine. The use of the values of the $\gamma_{\mu\nu}$ advanced by Mataga and Nishimoto gives almost as good agreement with the observed transition energies as do the original calculations by those authors. Thus, in summary, the variable electronegativity feature does not materially improve the

* The CI matrix elements were based on benzene orbitals as in the Parr-Pariser method but the exact geometry of pyridine was employed so that some of the geometrical simplifications made by Parr and Pariser were no longer valid.

† The dependence of the CIVESCF matrix elements on these quantities could have been determined directly, but it was considered that this more precise approach would not be worth the considerable computational labour involved.

agreement between the calculated and observed values. Mataga and Nishimoto's (1957) values of $\gamma_{\mu\nu}$ appear to give more reliable spectroscopic results than do the values obtained by the Pariser-Parr procedure, at least for heterocycles. The comparative insensitivity of the calculations to the VE modification is probably to be ascribed to the virtual cancellation of the effect when the energy difference between two states is evaluated. In Hückel MO parlance, the spectroscopic

TABLE 15

DEPENDENCE OF CI MATRIX ELEMENTS ON β_{CN} AND THE $\gamma_{\mu\nu}$

Element	Value (eV)
Singlets	
$(^1\psi_{2\to3} \mid \mathbf{H} \mid {}^1\psi_{2\to3})$	$5{\cdot}800-2\delta\beta/3-3\delta\gamma/72$
$(^1\psi_{1\to4} \mid \mathbf{H} \mid {}^1\psi_{1\to4})$	$6{\cdot}090-2\delta\beta/3+45\delta\gamma/72$
$(^1\psi_{2\to3} \mid \mathbf{H} \mid {}^1\psi_{1\to4})$	$1{\cdot}054+5\delta\gamma/24$
$(^1\psi_{2\to4} \mid \mathbf{H} \mid {}^1\psi_{2\to4})$	$6{\cdot}231+\delta\gamma/24$
$(^1\psi_{1\to3} \mid \mathbf{H} \mid {}^1\psi_{1\to3})$	$6{\cdot}440-4\delta\beta/3-5\delta\gamma/72$
$(^1\psi_{2\to4} \mid \mathbf{H} \mid {}^1\psi_{1\to3})$	$-0{\cdot}980-15\delta\gamma/24$
Triplets	
$(^3\psi_{2\to4} \mid \mathbf{H} \mid {}^3\psi_{2\to4})$	$4{\cdot}133+7\delta\gamma/24$
$(^3\psi_{1\to3} \mid \mathbf{H} \mid {}^3\psi_{1\to3})$	$4{\cdot}015-4\delta\beta/3+29\delta\gamma/72$
$(^3\psi_{2\to4} \mid \mathbf{H} \mid {}^3\psi_{1\to3})$	$-0{\cdot}252+\delta\gamma/2$

calculations are insensitive to values of coulomb terms and depend mainly on resonance integrals. On the other hand, the improved agreement between calculated and observed dipole moments in pyrrole and similar molecules indicates that the VESCF method gives better results for ground-state properties.

The oscillator strengths for the first two transitions of pyridine are relatively small. The calculated value in both cases is given by the difference between two considerably larger components and the result obtained is very sensitive to the

TABLE 16

TRANSITION ENERGIES OF PYRIDINE FOR MODIFIED MATRIX ELEMENTS

Excited State	(i)	(ii)	Observed
$^1B_1$	4·86	5·06	4·90
$^1A_1$	5·48	5·62	6·38
$^1B_1$	6·75	6·95	7·07
$^1A_1$	6·97	7·22	
$^3A_1$	3·58	3·72	3·67

form of the molecular orbitals and the exact geometry of the molecule. The VESCF pyridine orbitals for method *AP* give a very small value of f for the first $^1B_1$ transition (Table 14). For methods *BP* and *BJ* a larger calculated oscillator strength is obtained, but still less than the observed value. The magnitude of f in this case depends largely on the difference between the matrix elements $(^1\psi_{2\to3} \mid \mathbf{H} \mid {}^1\psi_{2\to3})$ and $(^1\psi_{1\to4} \mid \mathbf{H} \mid {}^1\psi_{1\to4})$ (see Table 5) which is greater in the case of method *B* than of method *A*. (For benzene the theoretical oscillator

strength of the corresponding transition is zero, and these two matrix elements are equal.) This might roughly be attributed to the fact that method *B* for pyridine implies a greater departure from the benzene structure than does method *A* (cf. the variations in the $F_{\mu\mu}$ (Table 1) and in the π-electron densities (Table 7)). Thus the first $^1B_1$ state and the second $^3B_1$ state are predicted to be more nearly degenerate by method *AP* than by method *BP* (see Table 14); the corresponding states in benzene ($^1B_{2u}$ and $^3B_{2u}$) are calculated to be degenerate (Pariser 1956). However, for the second transition ($^1A_1 - ^1A_1$) the values obtained are all much lower than would be expected; for methods *BP* and *BJ* they are actually lower than the value for the first, experimentally weaker, transition. This is probably caused by the sensitivity of the calculated value to the form of the orbitals and molecular geometry, a difference in two comparable terms again being involved.

The oscillator strengths for the next two N→V transitions are represented by the sum of terms and so are not so sensitive to the form of the orbitals. The experimental value* for the third transition is in reasonable agreement with the values obtained by all methods. In general, the CIVESCF calculations of oscillator strengths appear to be no better than the calculations of Mataga and Nishimoto using the CISCF method.

Possibly one should not expect better agreement between theoretical and observed spectroscopic intervals and transition probabilities until (i) the interaction of the nitrogen *n*-electrons with the π-electrons is explicitly included in the calculations and (ii) a separate value of β_{CN} is used for each spectroscopic state and a separate VESCF calculation of the orbitals for each state is performed. Although some recent calculations have incorporated the *n*-electrons (Anno 1958), their inclusion in the CIVESCF calculations must await an investigation of the dependence upon Z_μ of the additional monocentric coulomb repulsion integrals which occur.

VIII. The Spectrum of Pyridinium

The results of calculations of spectroscopic properties of the pyridinium cation by the CIVESCF method are shown in Table 17. The agreement with

Table 17

π-π* spectral transitions in pyridinium

Excited State	*BP* ($\beta_{CN} = -2{\cdot}30$ eV) Energy	*f*	*CP* ($\beta_{CN} = -2{\cdot}50$ eV) Energy	*f*	*AP* ($\beta_{CN} = -2{\cdot}50$ eV) Energy	*f*	Observed Energy
$^1B_1$	4·43	0·09	4·63	0·07	4·49	0·04	4·8
$^1A_1$	5·11	0·04	5·15	0·01	5·23	0·12	5·5
$^1B_1$	6·98	0·70	6·87		6·93		
$^1A_1$	6·95	1·15	7·01		6·86		

* The observed value of *f* probably includes some contribution from Rydberg transitions and may also represent the combined oscillator strengths of two overlapping N→V transitions in this region.

experiment is not as good as it might be, but could undoubtedly be improved by using a still more negative value of β_{CN} and perhaps also by reducing the values of the nearest neighbour $\gamma_{\mu\nu}$ as discussed above for pyridine. The data in Table 16 demonstrate how sensitive are the values of f for the first two N→V transitions to the detailed form of the orbitals. Methods *AP* and *CP*, differing only in the procedure for evaluating core attractions, give very different values for these transitions.

IX. Acknowledgment

One of us (M.L.H.) gratefully acknowledges the award of a C.S.I.R.O. studentship, during the tenure of which the above work was done.

X. References

Anno, T. (1958).—*J. Chem. Phys.* **29**: 1161.
Bak, B., Hansen-Nygaard, Lise, and Rastrup-Andersen, T. (1958).—*J. Mol. Spect.* **2**: 361.
Bernstein, H. J, and Schneider, W. G. (1956).—*J. Chem. Phys.* **24**: 469.
Brown, R. D., and Harcourt, R. D. (1959).—*J. Chem. Soc.* **1959**: (in press).
Brown, R. D., and Heffernan, M. L. (1957).—*Aust. J. Chem.* **10**: 211.
Brown, R. D., and Heffernan, M. L. (1958).—*Trans. Faraday Soc.* **54**: 757.
Brown, R. D., and Heffernan, M. L. (1959*a*).—*Aust. J. Chem.* **12**: 319.
Brown, R. D., and Heffernan, M. L. (1959*b*).—*Aust. J. Chem.* **12**: 330.
Brown, R. D., and Heffernan, M. L. (1959c).—*Aust. J. Chem.* **12**: (in press).
De More, B. B., Wilcox, W. S., and Goldstein, J. H. (1954).—*J. Chem. Phys.* **22**: 876.
Higasi, K., Omura, I., and Baba, H. (1956).—*J. Chem. Phys.* **24**: 623.
Mataga, N. (1958).—*Z. phys. Chem. N.F.* **18**: 285.
Mataga, S., and Mataga, N. (1959).—*Bull. Chem. Soc. Japan* **32**: 521.
Mataga, N., and Nishimoto, K. (1957).—*Z. phys. Chem. N.F.* **13**: 140.
Mulliken, R. S., and Rieke, C. A. (1941).—*Rep. Prog. Phys.* **8**: 231.
McWeeny, R., and Peacock, T. (1957).—*Proc. Phys. Soc.* A **70**: 41.
Pariser, R. (1953).—*J. Chem. Phys.* **21**: 568.
Pariser, R. (1956).—*J. Chem. Phys.* **24**: 250.
Pariser, R., and Parr, R. G. (1953*a*).—*J. Chem. Phys.* **21**: 767.
Pariser, R., and Parr, R. G. (1953*b*).—*J. Chem. Phys.* **21**: 466.
Pople, J. A. (1953).—*Trans. Faraday Soc.* **49**: 1375.
Watanabe, K. (1957).—*J. Chem. Phys.* **26**: 543.

Note added in Proof.—Mataga and Mataga (1959) have recently studied the ultraviolet spectrum of the pyridimium cation by a CIVESCF method. They concluded that the NH bond is not purely covalent because they did not obtain good agreement between theory and experiment when a covalent NH bond was assumed. Their conclusions seem premature because they used the pyridine value for β_{CN} in their calculations. Our studies of pyrrole and the pyrrole anion (Parts II and III) however show that different values of this resonance integral are required to account for the respective spectra.

The study by Mataga and Mataga supports our conclusion that the VE modification scarcely alters spectroscopic predictions.

INFRA-RED SPECTRA OF URANYL COMPOUNDS*

I. URANYL NITRATES

By J. G. ALLPRESS† and A. N. HAMBLY‡

[*Manuscript received July* 7, 1959]

Summary

The infra-red spectra of the di-, tri-, and hexahydrates of uranyl nitrate and of anhydrous potassium uranyl nitrate have been recorded. The results differ significantly from those of Gatehouse and Comyns (1958) but do not permit a decision regarding the structure of the complex anions in these compounds.

I. INTRODUCTION

A number of systems containing uranium oxides show ranges of composition which could be explained in terms of the presence of two forms of the uranyl radical of different valence, UO_2^{2+} and UO_2^{+}. As a preliminary to the investigation of the infra-red spectra of such compounds we studied some of the typical systems containing the divalent uranyl radical, including three hydrates of uranyl nitrate and the anhydrous salt, potassium uranyl nitrate. The spectral data in the literature for these compounds were in an extremely confused state but, just as we were completing this study, a further paper (Gatehouse and Comyns 1958) appeared. These authors reviewed the previous studies and presented new spectra. The latter, while agreeing more closely with our observations than did previous records, showed some significant differences and some of these points of difference were used as the bases of arguments regarding the structures of the compounds.

II. EXPERIMENTAL

The spectra were observed with a Perkin-Elmer 112, single-beam, double-pass, spectrophotometer, calibrated as described previously (Dyall and Hambly 1958). The specimens were ground between glass plates (Crook and Taylor 1958) with medicinal paraffin or " Florube No. 1 " (Imperial Chemical Industries). Mulls of uranyl nitrate hexahydrate prepared in this way showed aggregation of the particles and formation of liquid droplets when kept between sodium chloride plates ; the spectrum changed at the same time. They were quite stable if the plates were first coated with a film of polythene, deposited by evaporation of a carbon tetrachloride solution. The use of pressed disks of the sample mixed with potassium chloride was quite unsatisfactory with these compounds.

* The experimental work was carried out in the Chemistry Department, University of Melbourne.

† Chemistry Department, University of Melbourne.

‡ Chemistry Department, University College, Canberra, A.C.T.

(i) *Uranyl Nitrate Hexahydrate.*—A sample of analytical reagent quality was checked by analysis and used without further purification (Found : U, 47·5%. Calc. for $UO_2(NO_3)_2.6H_2O$: U, 47·4%).

(ii) *Uranyl Nitrate Trihydrate.*—The finely ground hexahydrate was dried over concentrated sulphuric acid at room temperature and atmospheric pressure (Found : U, 53·1 ; H (micro), 1·4%. Calc. for $UO_2(NO_3)_2.3H_2O$: U, 53·1 ; H, 1·4%).

(iii) *Uranyl Nitrate Dihydrate.*—The trihydrate was dried *in vacuo* over phosphorus pentoxide until dehydration appeared to cease when the loss in weight was 0·394 g compared to the calculated value of 0·400 g (Found : U, 55·6%. Calc. for $UO_2(NO_3)_2.2H_2O$: U, 55·4%).

(iv) *Potassium Uranyl Nitrate.*—Equimolar amounts of uranyl nitrate hexahydrate and potassium nitrate were dissolved in concentrated nitric acid and the solution allowed to evaporate in a closed vessel containing dishes of concentrated sulphuric acid and potassium hydroxide. Yellow-green, strongly fluorescent crystals of the product were collected over a period of several weeks (Found : K (flame photometer), 7·8% ; loss in weight on ignition to potassium diuranate 32·7%. Calc. for $KUO_2(NO_3)_3$: K, 7·9% ; loss on ignition, 32·7%).

III. Results and Discussion

The important regions of the spectra are shown in Figure 1, and the full results are presented in Table 1. The differences between these spectra and those obtained by Gatehouse and Comyns are discussed below.

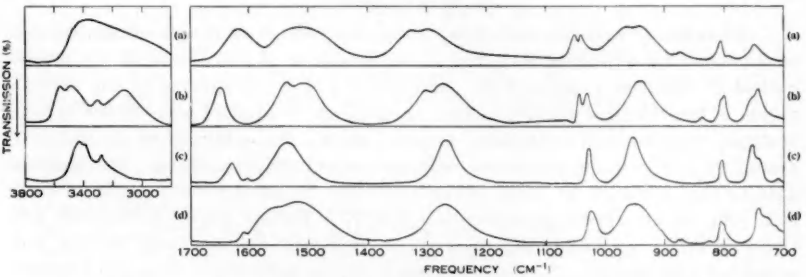


Fig. 1.—Infra-red spectra of (*a*) uranyl nitrate hexahydrate ; (*b*) uranyl nitrate trihydrate ; (*c*) uranyl nitrate dihydrate ; (*d*) potassium uranyl nitrate.

(*a*) *Uranyl Nitrate Hexahydrate*

Our spectrum, particularly in the region 1200–1600 cm^{-1}, seems to be much better defined than that of Gatehouse and Comyns, and does not conform to their assessment that it " is in most respects that of an ionic nitrate ". In previous papers (Gatehouse, Livingstone, and Nyholm 1957, 1958) the criterion for the recognition of coordinated nitrate groups has been the presence of absorption bands at about 1300 and 1500 cm^{-1}, while ionic nitrates were identified by the presence of absorptions at 1390, 830, 720 cm^{-1} with, occasionally, the " symmetric " motion absorbing weakly at 1050 cm^{-1} in solid compounds. Gatehouse and Comyns base their conclusion regarding the ionic structure of uranyl nitrate hexahydrate on the presence, in their spectrum, of an extremely broad band at 1366 cm^{-1} and a band of medium intensity at 1030 cm^{-1} which does not shift on isotopic replacement of ^{14}N by ^{15}N. These bands are identified with the doubly degenerate, asymmetric, and the symmetric, stretching motions

of the nitrate ion. They also find a band of medium intensity at 835 cm^{-1} which they attribute to the out-of-plane deformation of the nitrate ion while Sacconi, Caroti, and Paoletti (1958) find a similar band at 836 cm^{-1} which they believe to be the symmetric stretching mode of the uranyl ion. When we examined the dispersion of hexahydrate in medicinal paraffin, without protecting the sodium chloride plates with polythene, we also found a strong sharp peak at 836 cm^{-1} and a broad peak of medium intensity at 1032 as in the spectra previously reported. These can be attributed to the displacement of nitrate groups and water by interaction with the sodium chloride.

When the windows are properly protected we find that our specimen absorbs at 1297, 1333 cm^{-1} and in a broad double band 1497, 1536 cm^{-1}, while the band at 1030 cm^{-1} is replaced by a clearly resolved, double band 1042, 1052 cm^{-1}. The band at 835 cm^{-1} is not present though the band at 809 cm^{-1} which is quite weak in the spectrum of Gatehouse and Comyns is of medium strength in our

TABLE 1

INFRA-RED SPECTRA OF URANYL NITRATES

s, Strong; m, medium; w, weak; vw, very weak intensity; (sh), shoulder; (br), broad

Uranyl Nitrate			Potassium Uranyl Nitrate (cm^{-1})	Probable Assignment
Hexahydrate (cm^{-1})	Trihydrate (cm^{-1})	Dihydrate (cm^{-1})		
750 w	744 m 751 w(sh)	707 vw 743 w(sh) 752 s	715 vw(sh) 742 s 752 w	ν_3 and/or ν_5* ONO_2^-
788 vw 809 m	800 m 804 w(sh)	803 s	802 s(sh)	ν_6 ONO_2^-
869 w 874 w	837 —	— —	824 w 873 w 877 w	? $\nu_1 UO_2^{2+}$
937 s 964 s	943 s	952 s	953 s	$\nu_3 UO_2^{2+}$
1042 m 1052 m	1032 m 1045 m	1029 s	1025 s	ν_2 ONO_2^-
1297 m 1333 m	1276 s 1306 s	1268 s	1271 s	ν_1 ONO_2^-
1497 s 1536 s	1506 s 1540 s	1515 s 1540 s	1521 s 1555 s	ν_4 ONO_2^-
		1605 w	1608 w	$2 \times \nu_6$ ONO_2^-

* The numbering of the vibrations of the coordinated nitrate group follows that of Gatehouse and Comyns.

TABLE 1 (*Continued*)

Uranyl Nitrate			Potassium Uranyl Nitrate (cm^{-1})	Probable Assignment
Hexahydrate (cm^{-1})	Trihydrate (cm^{-1})	Dihydrate (cm^{-1})		
1621 s	1651 s	1640 s	—	H_2O deformation
	1735 w	1734 w	1733 w	$\nu_3UO_2^{2+}+\nu_6\,ONO_2^-$
1789 m	1776 w 1791 w	1771 w	1766 w	$\nu_2+\nu_5\,ONO_2^-$
	1818 w		1818 w	$\nu_1+\nu_3\,UO_2^{2+}$
	1976 vw	1974 w	1968 w 1989 w	$\nu_3UO_2^{2+}+\nu_2\,ONO_2^-$
2030 vw	2058 w	2046 w	2046 w	$2\nu_2\,ONO_2^-$
	2083 w 2286 w	2270 w	2270 w 2300 w	$\nu_4+\nu_5\,ONO_2^-$
2427 w	2526 w	2522 w	2520 w	?
2442 w	2552 w	2553 w	2546 w	$2\nu_1\,ONO_2^-$
			3027 w	$2\nu_4\,ONO_2$
	3124 s(br) 3301 m(br)	3280 m		HOH, H bonded
3496 s (vbr)	3496 s 3569 s	3393 s 3437 s		H_2O stretch

spectrum. Throughout, there is a strong resemblance between our spectra of the hexahydrate and trihydrate and we do not see any grounds for the conclusion that they have radically different structures. We agree with Gatehouse and Comyns in identifying the weak pair at 869, 874 cm^{-1} as the "symmetric" vibration of the uranyl ion.

(*b*) *Uranyl Nitrate Trihydrate*

Our spectrum of this compound corresponds closely with that of Gatehouse and Comyns except that the band at 800 cm^{-1} is just resolved into two members.

(*c*) *Uranyl Nitrate Dihydrate*

In preparing dispersions of this hygroscopic compound it is necessary to work very rapidly if it is not to become contaminated with the trihydrate. All previously published spectra of this compound are in fact those of a mixture of

the dihydrate and the trihydrate. The spectrum recorded by Gatehouse and Comyns corresponds to that obtained in our first trials, but by restricting the exposure of the sample it was found possible to make a dispersion in which the intensities of the bands at 1045 and 1306 cm^{-1} were reduced almost to zero intensity. We cannot agree with a decision on the structure of the dihydrate which is based on the presence of these absorptions. The splitting reported for the deformation frequency of the water molecule is also attributable to the presence of two hydrates.

(*d*) *Potassium Uranyl Nitrate*

Gatehouse and Comyns did not study this compound but investigated the closely related rubidium uranyl nitrate for which Hoard and Stroupe (1949) derived a structure from X-ray diffraction. In this structure three nitrate groups were distributed as bidentate ligands in a plane at a right angle to the uranyl group and Gatehouse and Comyns use the resemblance of the spectra of the nitrate vibrations in the anhydrous double nitrate and uranyl nitrate dihydrate to infer that the nitrate groups are also in a bidentate coordination in the latter. This resemblance is even more pronounced in our spectra though we found absorption by the uranyl symmetrical vibration which would not be expected if the $[UO_2(NO_3)_3]^-$ ion had the symmetrical structure suggested by Hoard and Stroupe. It is also surprising that the change from a unidentate to a bidentate ligand should not change the nitrate frequencies by a greater amount. These all remain in the ranges covered by nitrate absorptions in situations where single linkage only is possible. It is our opinion that the infra-red spectra do not give sufficient evidence on which to base structures such as those suggested by Gatehouse and Comyns, but, except in the case of the hexahydrate and the dihydrate, there is nothing in our spectra that is not consistent with their structures. For the dihydrate the absence of splitting of the planar ONO_2^- modes favours their formula III while the hexahydrate apparently contains coordinated nitrate ions.

(*e*) *Hydrate Vibrations*

The vibrations of the water molecules do not conform with the predictions of Sartori, Furlani, and Damiani (1958) for firmly bound ligands. Unfortunately, owing to the presence of a strong Christiansen distortion of the hydrogen stretching band of the hexahydrate, no structure was resolved. The stretching bands are well resolved for the other two hydrates and for the deformation vibrations of all three hydrates. Sartori predicts a rise in the deformation frequency to about 1700 cm^{-1} and a depression of the stretching frequencies to 3000–3100 cm^{-1}. Only the trihydrate shows any evidence for such a change.

We conclude that all the spectra provide evidence for the coordination of nitrate groups in uranyl nitrates.

IV. Acknowledgment

The award of a studentship to one of us (J.G.A.) by the Commonwealth Scientific and Industrial Research Organization is gratefully acknowledged.

V. References

Crook, A., and Taylor, P. J. (1958).—*Chem. & Ind.* **1958**: 95.

Dyall, L. K., and Hambly, A. N. (1958).—*Aust. J. Chem.* **11**: 513.

Gatehouse, B. M., and Comyns, A. E. (1958).—*J. Chem. Soc.* **1958**: 3965.

Gatehouse, B. M., Livingstone, S. E., and Nyholm, R. S. (1957).—*J. Chem. Soc.* **1957**: 4222.

Gatehouse, B. M., Livingstone, S. E., and Nyho[illegible], R. S. (1958).—*J. Inorg. Nucl. Chem.* **8**: 75.

Hoard, J. L., and Stroupe, J. D. (1949).—In "Spectroscopic Properties of Uranium Compounds." p. 36. (Eds. Dieke, G. H., and Duncan, A.) (McGraw-Hill Book Co.: New York.)

Sacconi, L., Caroti, G., and Paoletti, A. (1958).—*J. Inorg. Nucl. Chem.* **8**: 93.

Sartori, G., Furlani, C., and Damiani, A. (1958).—*J. Inorg. Nucl. Chem.* **8**: 119.

THE INTENSITY OF THE HYDROXYL BAND IN INFRA-RED SPECTRA

By T. D. Flynn,* R. L. Werner,* and (in part) B. M. Graham*

[*Manuscript received May* 3, 1959]

Summary

Values are reported for the molar integrated absorption intensity of 37 alcohols and phenols dissolved in carbon tetrachloride and measured at the fundamental stretching vibration of the O—H group.

Current methods of measuring the intensity are reviewed and a particular procedure recommended in the case of hydroxyl compounds.

The effect of various substituent groups on the absorption intensity is discussed, and correlation found between absorption intensity, peak frequency, pK_a value, and Hammett's σ value.

I. Introduction

It is now well established that the measured infra-red absorption intensity of certain characteristic functional groups is influenced by both the nature and location of other functional groups in the molecule. The magnitude of such differences in intensity has been shown by several authors (Barrow 1955 ; Brown 1957) to be proportionally far greater than similar well-known differences in peak frequency.

A thorough understanding of the reasons for such differences is obviously necessary before attempting to use accurate intensity measurements in either quantitative or structural analyses.

Similarly, a systematic study of groups of compounds containing a particular functional group should lead to a better understanding of the relative magnitude of the various substitution effects such as mesomeric effects, inductive effects, and hybridization effects. Unfortunately very few systematic studies appear to have been carried out, even of the key absorption bands. Compilation of such data has no doubt been handicapped considerably by lack of agreement between different authors on the actual mechanics of measuring the intensity. This confusion is probably due in part to the fact that not all the observed bands appear to have the same shape, and hence no one equation has yet been developed to fit all the observed absorption bands. Further, many measurements have been made under undesirably low resolution.

The experimentally observed intensity (A) of an absorption band is defined by the expression

$$A = 2{\cdot}303 \int \varepsilon_\nu \mathrm{d}\nu = \frac{1}{Cl} \int \log_{10} \left(\frac{I_0}{I} \right)_\nu \mathrm{d}\nu,$$

* Department of Physical Chemistry, University of New South Wales, Broadway, Sydney.

the integral being measured over the entire absorption band, where ε_ν is the molar extinction coefficient at frequency ν, C the concentration (in moles/litre), l, cell length (in cm), I, I_0 are the transmitted and incident intensities of monochromatic radiation of frequency ν.

For a single isolated band therefore, the absorption should diminish to zero at an infinite distance from the band maximum. In practice, as will be shown later, the absorption curve does not always gradually approach the zero-absorption line. With certain compounds the band appears to join the zero-absorbance line as little as 25 cm^{-1} from the band centre, while in others the wings of the curve flatten out well above the zero absorbance line and show no further tendency to diminish. The problem then is to decide the actual area to be measured.

This paper summarizes an attempt to establish a mathematically sound method of measuring the infra-red intensities of hydroxyl groups in solution; and records the intensities obtained for 37 compounds dissolved in CCl_4, measured at the fundamental stretching vibration of the O—H group near 3635 cm^{-1}.

The hydroxyl band was chosen for the present study since: (i) this band is reasonably far removed from likely overlapping bands; (ii) little is known of the effect of various substituents on the "free" O—H band in very dilute solutions; and (iii) hydroxyl bearing compounds are, in general, relatively easily prepared and purified, and are sufficiently soluble in suitable solvents such as carbon tetrachloride.

II. Experimental

The spectra were determined on a Perkin-Elmer single-beam double-pass spectrometer using a lithium fluoride prism. The computed spectral slit width was 4·6 cm^{-1}, chosen to hold the noise level to less than ½ per cent. full-scale deflection. The samples used were the purest available, the minimum standard of purity being analytical reagent grade taken from previously unopened containers. The carbon tetrachloride used was fractionated immediately before use.

ortho-Substituted phenols were excluded from the present study due to intramolecular hydrogen bonding which is known to persist even at extreme dilutions.

A study of the Beer's law plot of absorbance *v.* concentration indicated that for aliphatic alcohols association begins at a concentration around 0·017M, and for the phenols, around 0·005M. Four concentrations of each sample were examined below these limits, two obtained by direct weighing and two by dilution.

Special precautions were taken to prevent evaporation losses with three of the lower molecular weight extremely volatile alcohols (methanol, ethanol, and *iso*propanol) which, as Saier and Coggeshall (1948) point out, can show extreme volatility in dilute solution, due to the fact that the strong hydrogen bonding of the liquid state has been broken, permitting the compounds to show the volatility expected from their low molecular weights. Dry air was pumped continuously through the system to keep background water vapour absorption bands at a minimum. The residual water vapour bands were used as an internal check on the wavelength scale which was calibrated against the accurate data of Sleator (1918).

The cells used were specially selected fused silica cells of liquid thickness 0·50 and 1·00 cm, the same cell being used in every case for both solvent and solution scans.

Molar integrated intensities were determined from the plot of measured intensity against molar concentration. Ramsay's (1952) correction tables indicate that with computed spectral slit widths of 4·6 cm^{-1}, the correction for finite slit width is generally less than 2 per cent., that is, within the average uncertainty of results (±3 per cent.). The effect of finite resolving power on the intensity was therefore neglected.

All areas were measured by planimeter, measurements being repeated until successive determinations agreed to within one-half of 1 per cent.

III. Results

With the relatively high resolution available, it was noticed that the absorption band obtained could often be resolved graphically into two overlapping bands. Table 2 therefore lists four measured areas for each concentration of each alcohol. They are as follows:

Area No. 1 is the area remaining after graphically removing the secondary absorption band, measured to the zero-absorbance line over a distance of 50 wave numbers on either side of the peak frequency and corrected for the area under both wings of the curve out to infinity using Ramsay's (1952) method. Area No. 2 is the total area under the absorption curve measured as for area 1.

Table 1

Comparison of Half-Intensity Bandwidths

Compound	Brown and Rogers (1957) (cm^{-1})	Present Work (cm^{-1})	Presence of Secondary Band
tert.-Butyl alcohol ..	30	17	No
Methanol	39	21·5	Yes
sec.-Butyl alcohol	40	18·5	Yes
*cyclo*Hexanol	34	20	No
Benzyl alcohol	45	18	Yes

These areas have been included purely for comparison, which will be considered in Section IV; the observed curves are not Lorentzian.

Area No. 3 is the same as area 1 but measured only to the line joining the flat wings of the curves and hence containing no estimated wing correction. Area No. 4 is the same as area 2, but again measured only to the line joining the flat portions of the wings of the curves.

It will be obvious therefore that for those curves which do not contain a secondary absorption band, area 1 will be equal to area 2 and area 3 equal to area 4.

Table 3 lists the intensity of the major band, the intensity of the minor band (where applicable), the peak frequencies of both bands, and the intensity

TABLE 2

AREAS MEASURED FOR EACH ALCOHOL

All areas are stated in intensity units (I.U.), where 1 I.U. is 1×10^4 l mole^{-1} cm^{-2}

Alcohol	Area No. 1	Area No. 2	Area No. 3	Area No. 4
Primary Aliphatic				
Methanol	0·369	0·378	0·278	0·285
Ethanol	0·479	0·512	0·275	0·308
n-Propanol	0·392	0·510	0·283	0·343
n-Butanol	0·435	0·533	0·344	0·384
n-Amyl alcohol	0·432	0·495	0·309	0·364
n-Hexanol	0·392	0·481	0·279	0·348
n-Heptanol	0·378	0·495	0·275	0·341
n-Octanol	0·432	0·527	0·318	0·379
n-Decanol	0·443	0·542	0·285	0·352
n-Undecanol	0·512	0·595	0·323	0·369
n-Dodecanol	0·490	0·558	0·310	0·379
n-Tetradecanol	0·476	0·559	0·309	0·385
n-Hexadecanol	0·468	0·565	0·295	0·378
n-Octadecanol	0·492	0·570	0·298	0·372
Benzyl alcohol	0·409	0·595	0·265	0·342
*iso*Butyl alcohol	0·430	0·530	0·288	0·338
*iso*Amyl alcohol	0·471	0·548	0·300	0·373
Secondary Aliphatic				
*iso*Propanol	0·339	0·369	0·260	0·279
*cyclo*Hexanol	—	0·416	0·319	—
Tertiary Aliphatic				
tert.-Butyl alcohol (1)	—	0·260	0·192	—
tert.-Butyl alcohol (2)	—	0·264	0·198	—
tert.-Amyl alcohol	—	0·338	0·262	—
Phenols				
Phenol	1·166	1·166	0·976	0·976
o-Cresol	1·076	1·076	0·846	0·846
m-Cresol	1·104	1·104	0·892	0·892
p-Cresol	1·084	1·084	0·846	0·846
α-Naphthol	1·232	1·232	1·095	1·095
β-Naphthol	1·392	1·392	1·127	1·127
p-Chlorophenol	1·596	1·596	1·264	1·264
p-Bromophenol	1·476	1·476	1·248	1·248
o-Ethyl phenol	1·118	1·118	0·858	0·858
p-Ethyl phenol	1·341	1·341	1·161	1·161
m-Methoxyphenol	1·196	1·196	0·966	0·966
p-Methoxyphenol	1·156	1·156	0·968	0·968
p-(*tert.*-Amyl) phenol	1·148	1·148	1·040	1·040
2,3-Xylenol	1·144	1·144	0·968	0·968
2,4,6-Tri-(*tert.*-butyl) phenol	1·822	1·822	1·040	1·040
Unsaturated Aliphatic Alcohol				
Allyl alcohol	0·395	0·513	0·289	0·372

TABLE 3

MAJOR AND MINOR BANDS IN THE REGION OF THE OH FUNDAMENTAL

Alcohol	Identity No.	Intensity of Major Band in (I.U.)	Intensity of Minor Band in (I.U.)	Intensity of Minor Band as % of Total Band Area	Frequency of Major Band (cm^{-1})	Frequency of Minor Band (cm^{-1}) (estimated)
Methanol	1	0·278	0·007	2·5	3644·8	3668·1
Ethanol	2	0·275	0·04	13	3636·8	3627·0
n-Propanol	3	0·283	0·06	17	3639·7	3626·7
n-Butanol	4	0·344	0·05	14	3638·7	3626·2
n-Pentanol	5	0·309	0·06	17	3639·8	3626·6
n-Hexanol	6	0·279	0·07	20	3639·7	3626·0
n-Heptanol	7	0·275	0·07	20	3639·1	3626·5
n-Octanol	8	0·318	0·06	16	3640·1	3626·7
n-Decanol	9	0·285	0·07	18	3639·5	3626·5
n-Undecanol	10	0·323	0·05	14	3640·4	3626·5
n-Dodecanol	11	0·310	0·07	18	3641·0	3627·5
n-Tetradecanol ..	12	0·309	0·08	21	3639·3	3625·0
n-Hexadecanol	13	0·295	0·08	21	3639·9	3625·8
n-Octadecanol	14	0·298	0·07	19	3639·5	3626·5
*iso*Butyl alcohol ..	—	0·288	0·05	15	3642·2	3628·0
*iso*Amyl alcohol ..	—	0·300	0·07	20	3639·7	3626·3
*iso*Propanol	—	0·260	0·002	7	3628·5	3614·1
Allyl alcohol	—	0·289	0·08	19	3622·3	3639·1
Benzyl alcohol ..	—	0·265	0·08	21	3619·3	3637·9
→						←
tert.-Butyl alcohol (1)	—	0·192	—	—	3618·5	—
tert.-Butyl alcohol (2)	—	0·198	—	—	3618·0	—
tert.-Amyl alcohol ..	—	0·262	—	—	3620·0	—
Phenol	15	0·976	—	—	3612·5	—
o-Cresol	16	0·846	—	—	3615·3	—
m-Cresol	17	0·892	—	—	3614·4	—
p-Cresol	18	0·846	—	—	3615·8	—
α-Naphthol	19	1·095	—	—	3610·4	—
β-Naphthol	20	1·127	—	—	3609·5	—
o-Ethyl phenol ..	21	0·858	—	—	3615·0	—
p-Ethyl phenol ..	22	1·161	—	—	3614·2	—
p-Chlorophenol ..	23	1·264	—	—	3610·5	—
p-Bromophenol ..	24	1·248	—	—	3610·1	—
m-Methoxyphenol ..	25	0·966	—	—	3612·7	—
p-Methoxyphenol ..	26	0·968	—	—	3618·3	—
p-(*tert.*-Amyl) phenol ..	27	1·040	—	—	3615·3	—
2,4,6-Tri(*tert.*-butyl) phenol	28	1·040	—	—	3650·0	—
2,3-Xylenol	29	0·968	—	—	3618·3	—
*cyclo*Hexanol	—	0·319	—	—	3625·0	—

→ No subsidiary absorption band could be detected in the compounds below this rule.

of the minor band as a percentage of the total band intensity. All areas listed are stated in intensity units (I.U.) where one intensity unit is 1×10^4 l mole^{-1} cm^{-2}.

For reasons presented in Section IV, area No. 3 is the area to be used in comparing molar absorption intensities of different alcohols.

IV. Discussion

(*a*) *Overlapping Absorption*

In 19 of the 37 compounds examined the hydroxyl band was found to be complex, consisting of a main band together with a subsidiary absorption which appeared as a shoulder some 20 cm^{-1} from the peak of the main band. No

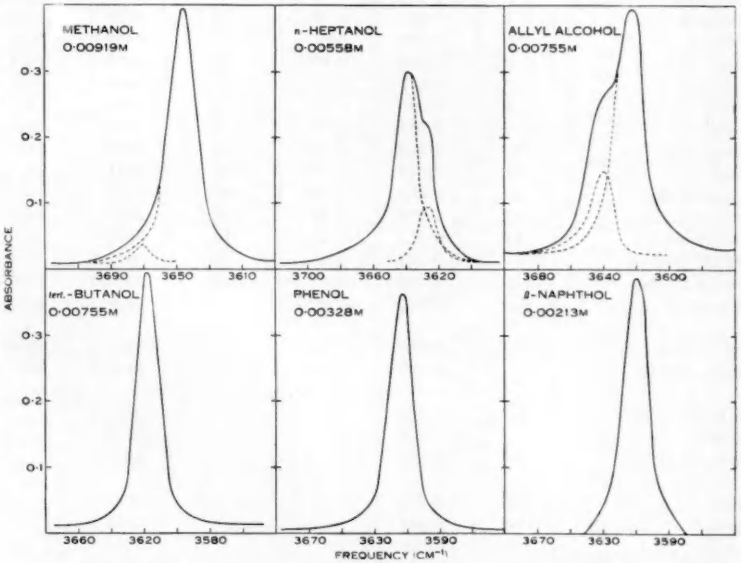


Fig. 1.—Infra-red absorption curves of some typical hydroxy compounds (cell length 1 cm).

question of hydrogen bonding arises since the contribution of this subsidiary band to the total area was independent of concentration and further, the band appeared in a few cases on the high frequency side of the main peak.

It appears that most previous workers have failed to detect this band because of the lower resolution used but its presence accounts for certain peculiarities in published data. For example, the unexplained variation in half-intensity bandwidths reported by Brown and Rogers (1957) can be traced to the influence of the subsidiary band as shown in Table 1.

Fox and Martin (1940*a*, 1940*b*) observed two overlapping bands in the spectrum of benzyl alcohol; a major band at 3617 cm^{-1} and a minor band at 3636 cm^{-1}. Their suggestion that the two bands represent firstly, molecules in which there is an interaction between the phenyl group and the O—H group

and secondly, molecules in which the two groups are well apart, is not supported by the present observation of a similar complex band structure with compounds such as ethanol having only one functional group.

Consideration of the curves obtained suggests that the subsidiary absorption band occurs only where the oxygen of the O—H group is attached to an (—R—CH_2—) group, and this band is therefore believed to be a combination band of the (—CH_2—O) group activated by the proximity of the strongly polar hydroxyl group. The fact that the subsidiary band is found to be stronger the closer that band is to the fundamental, is likewise suggestive of Fermi resonance which would involve an enhancement of intensity by a minor band near a major band. For the purposes of the present study it must be assumed that any such interaction does not appreciably change the O—H band intensity.

This assumption is probably justified since the subsidiary band never contributes more than 20 per cent. to the total band intensity.

(*b*) *Measurement of Band Areas*

As suggested in Section I the observed absorption curves for the O—H group seldom take the shape predicted of the Lorentz type function proposed by Ramsay (1952). With the more dilute solutions of phenols, the observed curve cuts the zero-absorbance line as little as 25 cm^{-1} from the band centre, while in more concentrated solutions of phenols and in almost all concentrations of aliphatic alcohols the wings of the curve do not gradually approach the zero absorbance line, but flatten out at a finite distance above it showing no further tendency to diminish, even as much as 100 cm^{-1} from the band centre. Some typical curves are reproduced in Figure 1.

A closer study of these curves reveals the following:

(i) The area beneath the wings of the curve is negligible in compact molecules such as the phenols, but may contribute up to 20 per cent. to the total band area in "bulky" molecules such as the long-chain aliphatic alcohols.

(ii) The area between the flat rings of the curve and the zero base line increases with increasing molecular weight. If the area bounded by the flat wings of the curve and the zero base line (area *A*) is plotted against molecular weight for the series methanol C_1 to octadecanol C_{18}, at the same molar concentration and over the same frequency range, a linear relationship with a small positive slope is obtained (Fig. 2). However, the area enclosed by the contour of the curve and a line joining the flat wings of the curve (area *B*) is found to be independent of molecular weight for the same series of compounds.

Ramsay found that, provided he assumed a Lorentz type function, then the area under the curve out to infinity was given by a constant, $\pi/2$, multiplied by the product of half-intensity bandwidth and peak absorbance. If, now, a similar relationship could be found between the area enclosed by the contour of the curve and a line joining the flat portions on the wings of the curve (area *B*) and the product of half-intensity bandwidth and peak absorbance for the series

of hydroxy compounds being examined, both aromatic and aliphatic (the constant K will no longer be $\pi/2$ for obvious reasons), it would seem reasonable to assume that this area, area B, is the true parameter and as such is the only area representing the specific absorption of the O—H group, that is, the absorption curves obtained in practice consist of the specific absorption curve of the hydroxyl group mounted on a general absorption curve representing such items as background absorption, Rayleigh scattering, and refraction losses due to differences in refractive index between solutions and pure solvent. For the 37 compounds examined, the value obtained for this constant K was $1{\cdot}205 \pm 0{\cdot}015$, a standard deviation of approximately 1 per cent. This is surprisingly good agreement considering that many of the curves were obtained after graphical separation of the subsidiary absorption band.

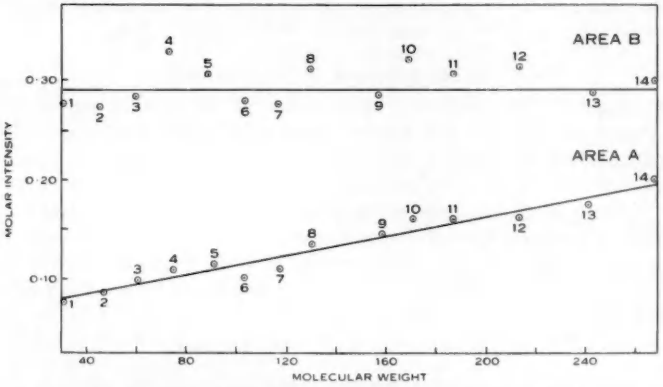


Fig. 2.—True integrated intensity (area B) and the area beneath the flat wings of the absorption curves (area A), as a function of molecular weight for the series of straight-chain aliphatic alcohols.

1, Methanol; 2, ethanol; 3, *n*-propanol; 4, *n*-butanol; 5, *n*-pentanol; 6, *n*-hexanol; 7, *n*-heptanol; 8, *n*-octanol; 9, *n*-decanol; 10, *n*-undecanol; 11, *n*-dodecanol; 12, *n*-tetradecanol; 13, *n*-hexadecanol; 14, *n*-octadecanol.

It is considered probable therefore, that values presented in the literature for the molar integrated intensity of various hydroxyl bearing compounds may be as much as 100 per cent. in excess of the true value, in that neither the overlapping subsidiary absorption bands undetected by low resolution, nor the general background absorption area will have been deducted from the measured intensity.

Tables 4 and 5 compare published values with those obtained in the present work for both aliphatic alcohols and phenols.

It will be noted that the spread of values is significantly less with the phenols due no doubt to the absence of secondary absorption.

Attempts to derive an equation covering the shape of the absorption band, which when integrated will yield an equation of the type

$$A = \frac{K}{Cl} \cdot \Delta\nu_{\frac{1}{2}} \cdot \log_e \left(\frac{I_0}{I}\right)_{\nu_{max.}},$$

with K equal to 1·205 have so far proved unsuccessful. Fox and Martin (1940*a*, 1940*b*) considered three feasible band shapes and predicted that K should be either 1·0, 1·138, or 1·57. Richards and Burton (1949) suggested a simple error function of the type

$$\log_e \left(\frac{I_0}{I}\right)_\nu = \log_e \left(\frac{I_0}{I}\right)_{\nu_{max.}} e^{-\beta(\nu-\nu_0)^2},$$

for several N—H and C=O bands in which K was 1·132. It is interesting to note that the error type functions of Richards and Burton (1949) and Willis

TABLE 4

COMPARISON OF PUBLISHED AND PRESENT VALUES FOR ALIPHATIC ALCOHOLS

Alcohol	Reference Number			Present Work
	1	15	4	
Methanol	0·53	0·334	0·54	0·278
Ethanol	0·62	—	—	0·275
n-Propanol ..	—	—	0·52	0·283
n-Butanol	0·72	—	—	0·344
sec.-Butanol ..	0·58	—	0·46	—
tert.-Butyl alcohol	0·42	—	0·39	0·196
*cyclo*Hexanol ..	—	—	0·44	0·319
Benzyl alcohol ..	—	—	0·66	0·265
Allyl alcohol ..	—	—	0·58	0·289

TABLE 5

COMPARISON OF PUBLISHED AND PRESENT VALUES FOR PHENOLS

Phenol	Reference Number			Present Work
	5	14	2	
Phenol	1·20	0·96	0·99	0·976
p-Bromophenol ..	—	—	1·23	1·248
p-Chlorophenol ..	—	1·1	1·19	1·264
p-Methoxyphenol ..	1·10	0·93	1·06	0·968
p-(*tert.*-Butyl) phenol	—	—	1·06	—
p-(*tert.*-Amyl) phenol	—	—	—	1·040
α-Naphthol ..	1·22	—	—	1·095
β-Naphthol ..	1·29	—	—	1·127
m-Methoxyphenol ..	—	0·98	—	0·966
p-Cresol	1·12	0·96	—	0·846

(1951) yield a closer approximation to the observed value of K than does the Lorentz type function proposed by Ramsay.

The position is, therefore, at present somewhat obscure. If subsequent studies of other key functional groups confirm that K is of the order of 1·2 for

all bands then it may be possible to derive an equation which will satisfactorily represent all infra-red absorption bands in solution, that is, it will then be possible to calculate the true molar integrated intensity of any band from measurements of peak height and half-intensity bandwidth at one concentration only, given sufficient resolution. The present availability of cheap replica gratings should allow such resolution to be attained.

The present results, however, confirm that below the association limit, the molar integrated absorption intensity of any hydroxyl bearing compound dissolved in carbon tetrachloride can be determined quite satisfactorily from measurements at one concentration only since the average uncertainty in K of ± 2 per cent. is significantly less than the reproducibility of successive determinations on the same alcohol sample (± 3 per cent.).

(c) *Comparisons of Observed Intensities*

Two significant features of the present results are firstly, the large differences in absorption intensity between the aliphatic alcohols and the phenols and secondly, the smaller but equally significant differences in intensity between different substituted phenols. It is interesting to note that whereas the peak frequencies of the aliphatic alcohols and phenols differ by less than 1 per cent. the intensities differ by as much as 300 per cent. The significant differences in intensity between the different substituted phenols suggests that it might be possible to identify and position a particular substituent group from the measured infra-red absorption intensity alone. Such information would be invaluable in structural organic analyses where the identification of particular hydroxyl bearing compounds has always proved difficult.

Several authors (Brown 1958 ; Krueger and Thompson 1959) have, in fact, attempted to demonstrate such a relationship between observed intensity and the nature of the substituent group.

For the series of aliphatic alcohols the most interesting feature is the constancy of intensity value. This is not altogether unexpected since in the restricted range of substituted aliphatic alcohols available for study, the small structural changes involved could not appreciably alter the sharing of the bonding and non-bonding electrons. Some interesting comparisons may, however, be drawn. For example, successive replacements of each hydrogen of the α carbon atom of methanol by the more strongly electropositive methyl group results in progressive decrease in infra-red intensity.

Alcohol :	Methanol	Ethanol	*iso*Propanol	*tert.*-Butyl Alcohol
Intensity (I.U.) :	0·278	0·275	0·260	0·196

The drop in intensity increases quite sharply with each successive hydrogen replacement and is certainly suggestive of the operation of dispersion type forces.

Similarly, as the point of entry of the methyl group is progressively removed from the hydroxyl the inductive effect of the methyl group rapidly diminishes and the intensity rises to the reasonably constant value of 0·29 I.U. for the remainder of the normal series.

It is with the substituted phenols, however, that the largest and most significant differences are found, due to the greater diversity of compounds available for study together with the operation of factors other than purely inductive effects, such as hybridization and mesomeric effects, overlap moments, and changes in partial ionic character. Attempts to estimate the relative contribution made by the various moments to the total bond moment of the vibration are handicapped by lack of available data, by the complexity of calculations, and above all by the interdependence of the various effects.

At present, therefore, one is restricted to a semi-empirical correlation of intensity with various other molecular properties.

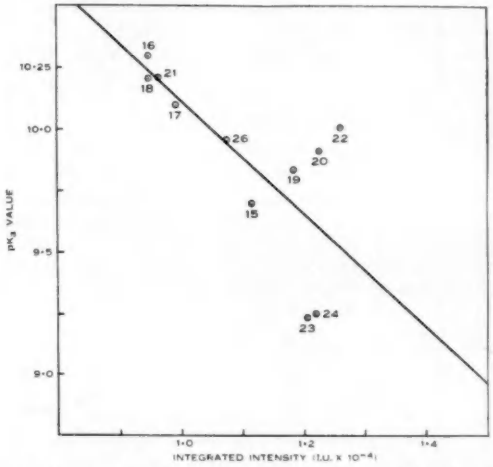


Fig. 3.—Integrated intensity of various substituted phenols as a function of pK_a value.
15, Phenol; 16, *o*-cresol; 17, *m*-cresol; 18, *p*-cresol; 19, α-naphthol; 20, β-naphthol; 21, *o*-ethyl phenol; 22, *p*-ethyl phenol; 23, *p*-chlorophenol; 24, *p*-bromophenol; 25, *m*-methoxyphenol.

The introduction of an electronegative substituent in the ring structure can, by promoting the formation of the three relatively unstable conjugated forms, decrease the electron density in the ring, thus increasing the polarity of the O—H band and hence increasing the measured infra-red intensity, the extent of the increase being a measure of the electronegativity of the particular substituent.

As expected, therefore, the more electronegative the substituent, the more polar will be the O—H band and hence the higher will be the measured infra-red absorption intensity. This is shown graphically in Figure 3. A simple linear relationship is found between pK_a value which is partially a measure of the polarity of the O—H bond, and measured infra-red intensity. At the same time the hybridization effect of a strongly electronegative substituent (namely,

halogens) in the para position, by causing delocalization of the unshared electron pairs of the oxygen atom, permitting them to enter the ring on the demand of the electronegative substituent, can cause a further polarization weakening of the O—H band beyond that normally expected from purely inductive effects, thus producing a further rise in infra-red absorption intensity.

The results obtained with the naphthols and with 2,4,6-tri(*tert.*-butyl) phenol are also significant. The naphthols, resonating between three stable Kekulé forms, each having approximately the same energy and hence contributing about equally to the normal state of the molecule, have a much higher resonance energy than phenol, that is, the O—H band will be more polar and the infra-red

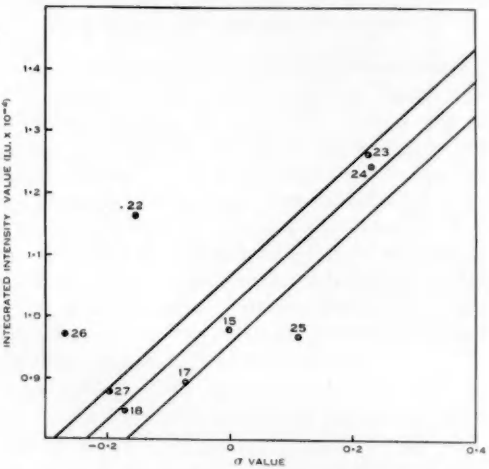


Fig. 4.—Integrated intensity of various substituted phenols as a function of Hammett σ value.
15, Phenol; 17, *m*-cresol; 18, *p*-cresol; 22, *p*-ethyl phenol; 23, *p*-chlorophenol; 24, *p*-bromophenol; 25, *m*-methoxyphenol; 26, *p*-methoxyphenol; 27, *p*-(*tert.*-amyl) phenol.

absorption intensity will be higher. Similarly, the observed difference in intensity between α- and β-naphthol is believed to be due to differences in charge separation and hence in structural stability. Some relation between the Hammett σ function and intensity can also be traced (Fig. 4).

The intensity obtained for 2,4,6-tri(*tert.*-butyl) phenol is similar to that obtained for phenol yet the peak frequency of 3650 cm^{-1} is higher even than that obtained for the aliphatic alcohols, and almost identical to values reported for the peak frequency of this compound in the vapour state, probably due to a combination of two factors, firstly, inhibition by the three bulky *tert.*-butyl groups of certain of the resonance structures of phenol and secondly, replacement of the solvent environment of the O—H group by an alkyl group environment. It may be, therefore, that while frequency is very sensitive to solvent interaction,

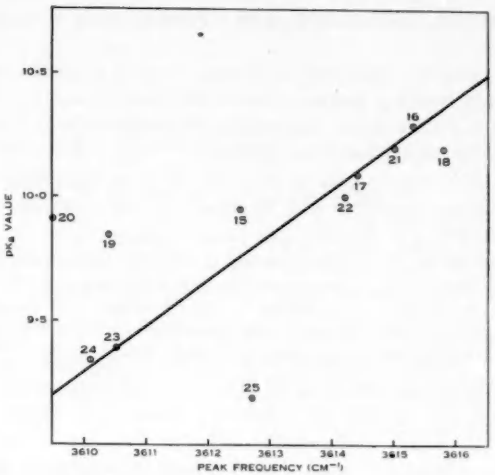


Fig. 5.—Peak frequency of various substituted phenols as a function of pK_a value.

15, Phenol; 16, *o*-cresol; 17, *m*-cresol; 18, *p*-cresol; 19, α-naphthol; 20, β-naphthol; 21, *o*-ethyl phenol; 22, *p*-ethyl phenol; 23, *p*-chlorophenol; 24, *p*-bromophenol; 25, *p*-methoxyphenol.

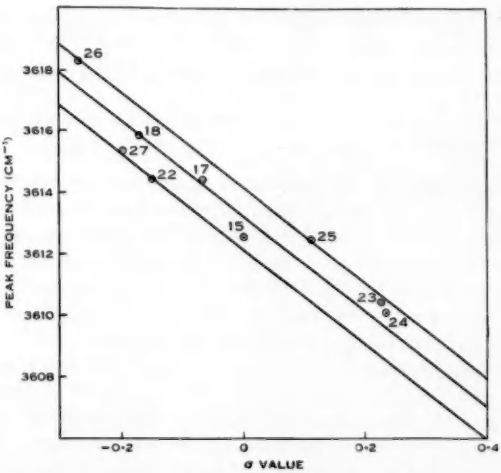


Fig. 6.—Peak frequency of various substituted phenols as a fun tion of Hammett σ value.

15, Phenol; 17, *m*-cresol; 18, *p*-cresol; 22, *p*-ethyl phenol; 23, *p*-chlorophenol, 24, *p*-bromophenol; 25, *m*-methoxyphenol; 26, *p*-methoxyphenol; 27, *p*-(*tert.*-amyl) phenol.

intensity is relatively independent of it. Further work is continuing on this point.

The results shown graphically in Figures 5 and 6 support firstly, the simple linear relationship between frequency and pK_a value found by Goulden (1954), and secondly, the simple linear relationship between frequency and Hammett σ value proposed by Ingraham *et al.* (1952).

Attempts have been made to establish both a logarithmic and a linear relationship between intensity and Hammett σ value (Stone and Thompson 1957; Brown 1958).

The present series of values covering a slightly wider range of value than those of Brown (1958) tend to support the linear relationship.

V. Acknowledgments

The authors desire to express their thanks to Professor A. E. Alexander of the University of Sydney and to the Chemical Spectroscopy Laboratory, Timbrol Limited, for the gift of laboratory samples of various alcohols and phenols.

VI. References

Barrow, G. M. (1955).—*J. Phys. Chem.* **59**: 1129.
Brown, T. L. (1957).—*J. Phys. Chem.* **61**: 820.
Brown, T. L. (1958).—*Chem. Rev. Lond.* **58**: 592.
Brown, T. L., and Rogers, M. T. (1957).—*J. Amer. Chem. Soc.* **79**: 577.
Flett, M. St. C. (1957).—*Spectrochim. Acta* **10**: 21.
Fox, J. J., and Martin, A. E. (1940*a*).—*Trans. Faraday Soc.* **36**: 889.
Fox, J. J., and Martin, A. E. (1940*b*).—*Trans. Faraday Soc.* **36**: 897.
Goulden, J. D. (1954).—*Spectrochim. Acta* **6**: 129.
Ingraham, L. L., Corse, J., Bailey, G. F., and Stitt, F. (1952).—*J. Amer. Chem. Soc.* **74**: 2297.
Kreuger, P. J., and Thompson, H. W. (1959).—*Proc. Roy. Soc.* A **250**: 22.
Ramsay, D. A. (1952).—*J. Amer. Chem. Soc.* **74**: 72.
Richards, R. E., and Burton, W. R. (1949).—*Trans. Faraday Soc.* **45**: 874.
Saier, L., and Coggleshall, N. D. (1948).—*Liebigs Ann.* **20**: 812.
Sleator, W. W. (1918).—*Astrophys. J.* **48**: 125.
Stone, P. J., and Thompson, H. W. (1957).—*Spectrochim. Acta* **10**: 17.
Tsubomura, H. (1956).—*J. Chem. Phys.* **24**: 927.
Willis, J. B. (1951).—*Aust. J. Sci. Res.* A **4**: 172.

THE INFRA-RED SPECTRA OF 2- AND 4-QUINOLONES

By J. R. Price* and J. B. Willis†

[*Manuscript received July* 9, 1959]

Summary

Examination of the infra-red spectra of thirty 2- or 4-quinolones shows that discrimination between them on the basis of the frequency of the carbonyl absorption band is not reliable. Certain quinolinyl ethers and quinolinium salts also show absorption in the same region of the spectrum as the quinolones. The spectra of several acridine derivatives have been measured for comparison with those of the quinolones.

I. Introduction

It is becoming accepted that 2- and 4-quinolones may be distinguished from each other by the frequency of the infra-red absorption band due to the vibration of the carbonyl linkage, 2-quinolones absorbing near 1660 cm^{-1} and 4-quinolones near 1630 cm^{-1} (Witkop, Patrick, and Rosenblum 1951 ; Grundon, McCorkindale, and Rodger 1955 ; Grundon and McCorkindale 1957). In a study of a number of 2- and 4-quinolones, some synthetic, others obtained in the course of structural studies of naturally occurring quinoline derivatives, the differentiation of the two types by infra-red spectroscopy has been further examined. The frequencies of absorption in regions relevant to the discussion are shown‡ in Table 1. The spectra of representative compounds, measured in Nujol mulls, are shown in Figures 1 and 2.

II. Discussion

(*a*) *The 1450–1700 cm^{-1} Region*

The spectra of most of the compounds studied are somewhat complex in this region, but the band having the highest frequency is believed to be due to the carbonyl stretching vibration, and it is the frequency of this band which is regarded as permitting discrimination between 2- and 4-quinolones. The data in Table 2 show the frequency ranges in which this band occurs for the different classes of quinolones studied. It will be seen that there is overlap between the frequency ranges for *N*-methyl-2-quinolones and for *N*-unsubstituted 4-quinolones, and that discrimination between these types of compounds on the basis of the frequency of this band would not be very reliable. Support for this view is provided by Goodwin, Shoolery, and Johnson (1959), who, in connection with the structure determination of the alkaloid lunine (VI), state that " no clear-cut decision could be made from the infra-red spectrum ; the medium

* Organic Chemistry Section, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

† Division of Chemical Physics, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

‡ We are fortunate in being able to include several spectra recorded for one of us (J.R.P.) some years ago by Dr. R. N. Haszeldine, and one spectrum recorded by Dr. R. G. Gillis.

TABLE 1

QUINOLONES : ABSORPTION FREQUENCIES IN 1450–4000 cm^{-1} RANGE

No.	Compound	State	Frequencies (cm^{-1})									
2-Quinolones												
1	2-Quinolone	Mull*†		3110			1653	1599		1559	1501	1471
							1640					
		$CHCl_3$ soln.	3350				1664	1609		1561		
2	4-Methyl-2-quinolone	Mull†‡		3110 sh			1660	1614 sh		1556	1509	
		$CHCl_3$ soln.	3330				1664	1612		1562	1510	
3	4,7 - Dimethoxy - 3 - (2′-hydroxyethyl)-2-quinolone	Mull	3400	3080			1647	1626 sh		1561	1509	
		$CHCl_3$ soln.	3400				1644				1513	
4	Structure II§	Mull		3160			1664	1602			1492	
5	4-Hydroxy-2-quinolone	Mull		3080		2500	1667	1637		1550	1508	
								1616				
								1597				
1-Methyl-2-quinolones												
6	1-Methyl-2-quinolone	Mull					1646	1585	1570 sh		1499	
7	4 - Hydroxy - 1 - methyl - 2 - quinolone	Mull				2500	1650	1600		1550	1515	
						1920					1499 sh	
8	4 - Methoxy - 1 - methyl - 2 - quinolone	Mull					1637	1619	1587		1505	
9	4,7 - Dimethoxy - 1 - methyl - 2-quinolone	Mull	3220		2735	2685	1642	1595	1568 sh			
		$CHCl_3$ soln.					1638	1608	1586			
10	4-Hydroxy-3-(2′-hydroxy-*iso*amyl) - 8 - methoxy - 1 - methyl-2-quinolone	Mull	3340		2705		1638	1608	1586		1495	
11	Lunacridine (I)	Mull	3370				1633	1611	1588			
		CCl_4 soln.	3400						1577			
		$CHCl_3$ soln.	3360				1635	1614	1586			
12	Acetyl lunacridine	Mull				1730	1645	1624	1580			
								1602				
		$CHCl_3$ soln.				1727	1643	1625 sh	1580			
								1599				
13	Structure IV	Mull					1666	1637	1572		1499	
								1603				
14	Structure III	Mull	3320				1657	1620	1575 sh			
								1594				

TABLE 1 (*Continued*)

No.	Compound	State	Frequencies (cm^{-1})									
4-Quinolones												
15	4-Quinolone	Mull	Broad absorption from 2200–3250 cm^{-1}				1637	1611	1593 1563 sh	1548	1508	1475
16	2-Methyl-4-quinolone	Mull	3290	3070	2812	2690 sh	1647	1602		1558	1508	1475
		$CHCl_3$ soln.					1638	1608				
17	3 - Ethyl - 2 - methyl - 4 - quinolone	Mull	3280	3090	2740		1642	1609	1593	1558	1508	
		$CHCl_3$ soln.					1635					
18	2-Phenyl-4-quinolone	Mull	3250	3100	2735		1638	1618 1599	1585	1549	1509	1477
19	7 - Methoxy - 2 - phenyl - 4 - quinolone	Mull	3240	3130	2670		1638	1614 1598	1580	1544	1521 1473	
20	7 - Methoxy - 3 - phenyl - 4 - quinolone	Mull	3210	3075	2650 sh		1636	1616 1593	1563		1518	
1-Methyl-4-quinolones												
21	1-Methyl-4-quinolone	Mull					1629	1616	1577	1550	1497 1477	
22	1 - Methyl - 2 - phenyl - 4 - quinolone	Mull					1620	1595	1573 sh	1550	1503 1488	
23	7 - Hydroxy - 1 - methyl - 2 - phenyl-4-quinolone	Mull			2600		1623	1608	1572		1522	
24	7 - Methoxy - 1 - methyl - 2 - phenyl-4-quinolone	Mull					1618	1605 sh	1585 sh 1575	1550 sh		
25	7 - Methoxy - 1 - methyl - 3 - phenyl-4-quinolone	Mull					1629	1603 sh	1575	1550 sh	1497 sh	1481 sh
26	Lunacrine (V)	Mull					1625	1598		1556	1515	
		CCl_4 soln.					1626	1599		1558	1517	
27	Lunine (VI)	Mull					1642 1627 sh	1589	1568	1550 sh	1520	1493
28	Dihydro*iso*acronycidine (VII)	Mull					1637		1586 1563	1542 sh		
29	*iso*Acronycidine	Mull					1633	1620	1590	1540	1514	1480 sh
30	Nor*iso*acronycidine (VIII)	Mull					1638		1590 1567 sh	1543 sh	1509 1489	

TABLE 1 (*Continued*)

No.	Compound	State	Frequencies (cm^{-1})									
Quinolines and Quinolinium Salts												
31	2-Methoxyquinoline	Liq.		3080			1621	1611	1593 sh 1575		1510	1488
32	4-Methoxyquinoline	Mull					1620	1599	1570		1515 sh 1508	
33	2,4-Dimethoxyquinoline	Mull					1625	1608	1580		1515	
34	Dihydroacronycidine (IX)	Mull					1625	1600 sh	1587	1540 sh	1512	1483 sh
35	4 - Methoxy - 1 - methyl quinolinium iodide	Mull					1617		1585 1565 sh	1539	1499	
36	Lunasine perchlorate (X)	Mull					1637	1605		1538	1495	
37	Methyl acronycidinium iodide (XI)	Mull					1637	1597	1583	1558 1533		

* The present measurements on solid 2-quinolone agree with those of Grundon, McCorkindale, and Rodger (1650, 1605, 1555, and 1505 cm^{-1}) and with those of Sheinker and Pomerantsev but differ slightly from those of Gibson, Kynaston, and Lindsey who record 1635, 1593, 1552, and 1500 cm^{-1}.

† Mull in tetrachloroethylene.

‡ The present measurements differ from those of Gibson, Kynaston, and Lindsey who obtained values of 1645, 1604 sh, 1554, and 1506 cm^{-1}.

§ Chemical evidence (Lamberton and Price 1953*b*) does not define whether the dihydrodimethylpyran ring is fused in the 5,6- or 6,7-positions. However, nuclear magnetic resonance spectral data (Goodwin, personal communication) have recently shown that it must be in the angular 5,6-position as shown in II. The infra-red spectral data is consistent with this conclusion, there being a band at 815 cm^{-1} which may be ascribed to the presence of two adjacent unsubstituted aromatic carbon atoms.

strong band at 6·09 μ was 0·09 μ lower than the corresponding band in lunacrine and would suggest the 2-quinolone class for lunine". These authors showed on other evidence that lunine is, in fact, a 4-quinolone. Moreover, certain compounds without quinolone functions but of types which might be encountered in studies of naturally occurring quinolone derivatives absorb in this region.

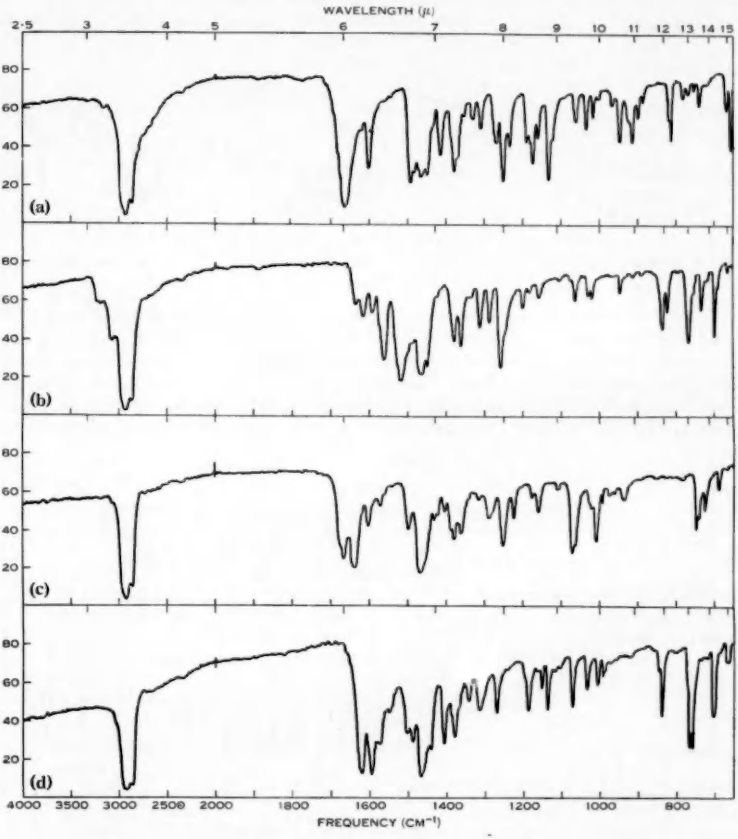


Fig. 1.—Infra-red absorption spectra of: (*a*) Structure II, compound No. 4; (*b*) 7-methoxy-4-quinolone, compound No. 20; (*c*) structure IV, compound No. 13; (*d*) 1-methyl-2-phenyl-4-quinolone, compound No. 22.

The frequency of the absorption due to a 2-quinolone carbonyl group (1633–1667 cm^{-1}) corresponds to that reported for 2-pyridone (1654 cm^{-1}; Mason 1957). Likewise the 4-quinolone carbonyl absorption (1618–1647 cm^{-1}) corresponds to that of 4-pyridone (1638 cm^{-1}; Mason 1957) and of a series of acridones (1626–1638 cm^{-1}, Table 3). Cook *et al.* (1957) find that the carbonyl

frequencies of alkyl-substituted 1-methyl-2-quinolones in carbon bisulphide solution range from 1634–1658 cm^{-1}. Their results indicate that the frequency is decreased by substituents in the heterocyclic ring but increased by substituents in the homocyclic ring. The present results, taken in conjunction with those of Grundon and of Witkop, do not indicate any very simple relationship between

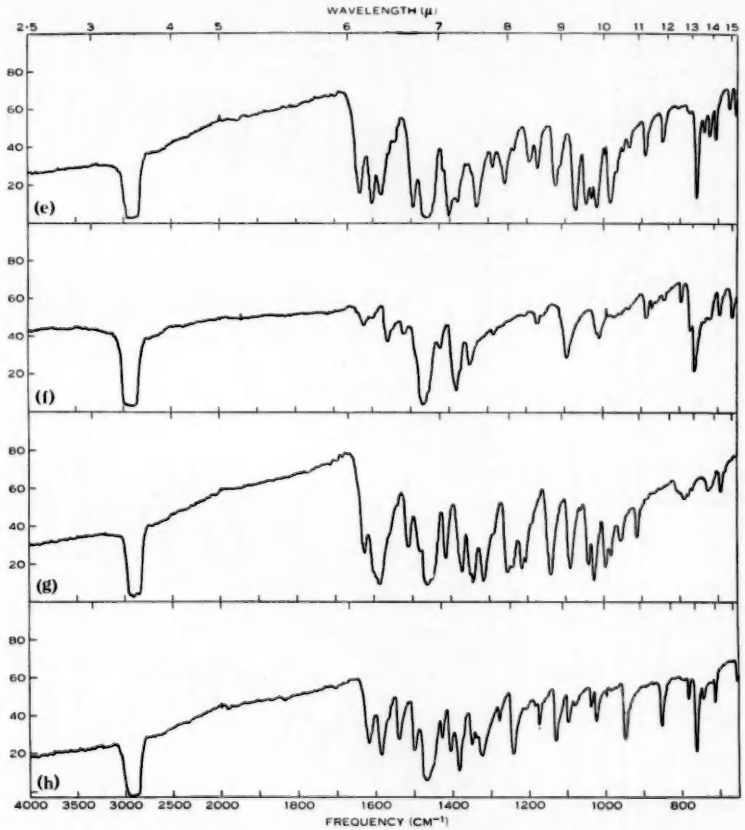


Fig. 2.—Infra-red absorption spectra of: (*e*) 1,2,3,4-tetramethoxy-10-methylacridone, compound No. 43; (*f*) 5-ethoxyacridine, compound No. 38; (*g*) dihydroacronycidine(VIII), compound No. 33; (*h*) 4-methoxy-1-methylquinolinium iodide, compound No. 34.

the nature of the substituent and the value of the carbonyl frequency. However, in 2-quinolones fused oxygen-containing rings in the 3,4-position raise the carbonyl frequency, while a hydroxyl group on a side-chain in the 3-position which can interact with the carbonyl group lowers it. This is exemplified by the spectra of the alkaloid lunacridine (I) and of its acetyl derivative; the band at 1634 cm^{-1}

TABLE 2

CHARACTERISTIC FREQUENCY RANGES FOR THE CARBONYL VIBRATION IN QUINOLONES

Type	Frequency Range (cm^{-1})	State	Authors
2-Quinolones	1647–1667	Mull	Present work
	1656–1661	$CHCl_3$ soln.	Witkop
	1641–1660*	Mull or KBr disk	Grundon
1-Methyl-2-quinolones	1633–1666	Mull	Present work
	1634–1658	CS_2 soln.	Cook *et al.*
4-Quinolones	1636–1647	Mull	Present work
	1626–1645	Mull	Witkop
	1620–1630	KCl disk	Grundon
1-Methyl-4-quinolones	1618–1642	Mull	Present work

* A compound believed by Grundon and McCorkindale (1957) to be 4-chloro-3-(2′-methoxyethyl)-8-methoxy-2-quinolone absorbs at 1631 cm^{-1}. However, the reported analysis of this compound agrees just as well with its formulation as the hemiacetal, 4-chloro-3-(2′-ethoxy-2′-hydroxyethyl)-8-methoxy-2-quinolone. If this structure, which seems more plausible from the method of preparation, is correct, the abnormally low frequency of 1631 cm^{-1} is accounted for by the interaction between the hydroxyl and quinolone carbonyl groups as discussed on pp. 593-4.

TABLE 3

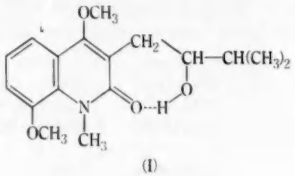
SPECTRA OF ACRIDINE DERIVATIVES MEASURED IN NUJOL MULLS

No.	Compound	Frequencies (cm^{-1})				
38	5-Methoxyacridine	1621	1604 sh	1563	1545 sh 1538 sh	1521 1485 sh
39	5-Ethoxyacridine	1620	1600 sh	1558	1550 sh 1537 sh	1517 1488 sh
40	5-Acridone	1632	1596	1570 sh	1550 1530	
41	2,4 - Dimethoxy - 10 - methylacridone	1634	1600	1561	1545 sh	1505
42	4 - Hydroxy - 2 - methoxy-10-methylacridone	1638	1592	1565 sh	1554	1512 1500 sh
43	4 - Methoxy - 2,3 - methylenedioxy-10-methylacridone	1628	1592	1574	1545 sh	1504 1480 sh
44	1,2,3,4 - Tetramethoxy - 10-methylacridone	1635	1603	1580	1545	1495
45	4-Hydroxy-1,2,3-trimethoxy-10-methylacridone*	1630	1615 sh	1589	1555 1545 sh	1500 1485 sh
46	1-Hydroxy-2,3,4-trimethoxy-10-methylacridone†	1626	1600 1590	1575	1555	1500

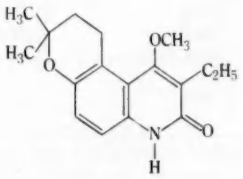
* This compound also shows a weak band at 2590 cm^{-1}.

† This compound also shows a band of medium intensity at 3200 cm^{-1}.

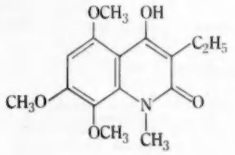
in the spectrum of I is displaced to 1644 cm^{-1} in the spectrum of acetyl lunacridine. On the other hand, the strong hydrogen bonding between a *peri*hydroxyl group and the carbonyl group of the acridones (Table 3) or *iso*furoquinolones (cf.



(I)

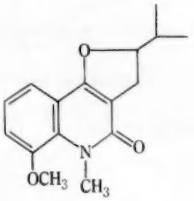


(II)

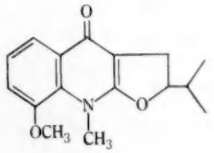


(III)

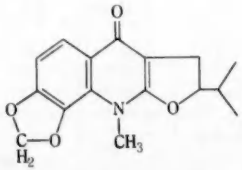
Table 1, Nos. 29 and 30) does not lead to displacement of the carbonyl frequency. This is closely analogous to the observed lack of frequency shift with 5-hydroxyflavones (Bellamy 1957) and, unlike the behaviour of the homocyclic *peri*-



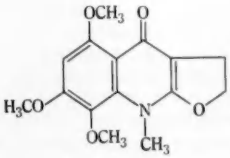
(IV)



(V)



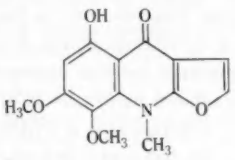
(VI)



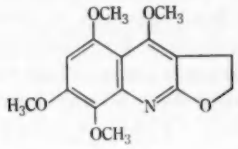
(VII)

hydroxyquinones, would appear to be associated with the negative character of the carbonyl oxygen arising from charge separation between it and the heteroatom.

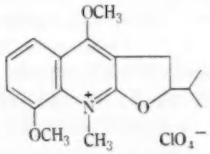
The remaining bands shown by the quinolones in this region, mainly between 1500 and 1600 cm^{-1}, are presumably due to " ring " vibrations involving C=C and C=N bonds ; the frequency pattern resembles that in the spectra of quinoline and some of its simple derivatives (Barnes *et al.* 1944 ; Bellamy 1958). Four quinoline derivatives (Nos. 31–34) containing methoxy groups in the 2- and/or 4-positions, but no quinolone function, show, in addition to absorption in the 1500–1600 cm^{-1} region, a strong band at 1620–1625 cm^{-1}, the region normally regarded as characteristic of the carbonyl vibration in 4-quinolones. Grundon and McCorkindale report similar absorption in the 1630–1645 cm^{-1} region for several quinoline derivatives with an ether function in the 2-position. However, the occurrence of " ring " vibrations at frequencies as high as 1620 cm^{-1} is not



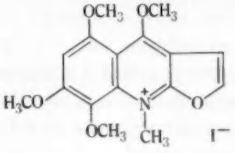
(VIII)



(IX)



(X)



(XI)

uncommon ; quinoline (Barnes *et al.* 1944), acridine (Cannon and Sutherland 1951), and alkoxyacridines (Table 3) absorb at this frequency, while bromoquinolines and quinolinium salts may absorb at even higher frequencies (Hambly personal communication). Three quinolinium salts (Nos. 35–37) absorb at 1617, 1637, and 1637 cm^{-1} respectively.

In general, the spectra of the 4-quinolones are more complex below 1600 cm^{-1} than are those of the 2-quinolones ; bands near 1550 and 1600 cm^{-1} are frequently split, and an additional band is often found near 1475 cm^{-1}.

(*b*) *The 2500–4000 cm⁻¹ Region*

(i) *Absorption due to >NH Groups.*—In agreement with the results of Gibson, Kynaston, and Lindsey (1955), the 2-quinolones examined show no absorption above 3160 cm^{-1} in the solid state, whereas the 4-quinolones show broad absorption in the 2500–3300 cm^{-1} region, which suggests that the N–H . . . O bonds are short and highly polar.

Absorption near 2800 cm^{-1} due to the presence of the >N–CH_3 group, as discussed by Braunholtz *et al.* (1958), was not observed in any of the 1-methyl-2-

or 4-quinolones or of the *N*-methylacridones studied. This is in agreement with the conclusion of these authors that absorption at this frequency depends on the nitrogen atom retaining its lone pair of electrons, which, because of the polar character of the quinolones and acridones, it evidently does not.

(ii) *Absorption due to the –OH Group in Hydroxy Derivatives of Quinolones.*—Three 2-quinolones (Table 1, Nos. 3, 10, and 11) containing a hydroxyl group in a side-chain were examined, the hydroxyl group in each being in such a position that hydrogen bonding to the quinolone carbonyl group is sterically possible with the formation of a 7-membered ring. In each spectrum a sharp, intense band occurs between 3340 and 3400 cm^{-1}. When two of these compounds (Nos. 3 and 11), whose solubilities permitted, were examined in chloroform solution the band appeared at almost the same frequency as in the solid state, which suggests that the hydroxyl group is intramolecularly bonded to the quinolone carbonyl group. This view is supported by the carbonyl stretching frequency being about 10 cm^{-1} lower than in related molecules in which there is no such hydroxyl group. Compound No. 10 also contains a hydroxyl group in the 4-position of the heterocyclic ring, and the band at 3340 cm^{-1} is broader than in the other two compounds. The bonding in this compound is probably partly intermolecular.

Compounds Nos. 5, 7, and 23, containing a hydroxyl group as a ring substituent, do not show absorption in the usual "hydroxyl region" but instead show broad absorption between 2500–2700 cm^{-1}. Compound 10, which also contains a hydroxyl group in a side-chain, absorbs in both regions. On the other hand, in the spectrum of compound No. 14 (III) the hydroxyl absorption appears at 3320 cm^{-1}, which suggests that the *o*-ethyl and *peri*methoxyl groups adjacent to the hydroxyl group and the ethyl and *N*-methyl groups adjacent to the quinolone carbonyl interfere with strong intermolecular hydroxyl-carbonyl interaction. The absorption between 2500 and 2700 cm^{-1} shown by the other four hydroxy compounds indicates the existence of hydrogen bonding stronger than any except that found in keto-enol systems. 4-Hydroxy-2-quinolones, of course, bear a close structural resemblance to β-diketones, but cannot form the strong intramolecular bond characteristic of such aliphatic systems. A similar displacement of hydroxyl frequency to 2430 cm^{-1} was observed in the spectrum of 3-hydroxypyridine by Gibson, Kynaston, and Lindsey (loc. cit.) and attributed by them to the formation of intermolecular OH . . . O and OH . . . N bonds.

III. Experimental

(*a*) *Compounds Nos. 1, 2, 5, 6, 7, 8, 9, 15, 16, 17, 21, 31, 32, 33, 38, 39, and 40.*—These were either purified commercial specimens or were prepared by standard methods. Compound No. 3 was kindly supplied by Mr. R. G. Cooke see Cooke and Haynes (1958). For compound No. 4 see Lamberton and Price (1953*b*), compounds Nos. 10, 11, 12, 13, 35, 36, and 37 see Price (1959), compounds Nos. 18, 19, 20, 22, 23, 24, 25, 26, and 27 see Johnstone, Price, and Todd (1958), compounds Nos. 29, 30, and 34 see Lahey, Lamberton, and Price (1950), compounds Nos. 41 and 42 see Lamberton and Price (1953*a*), compounds Nos. 44, 45, and 46 see Crow and Price (1949*a*, 1949*b*), and compound No. 43 see Hughes and Neill (1949).

(*b*) *3-Ethyl-4-hydroxy-1-methyl-5,7,8-trimethoxy-2-quinolone* (*(III) : Compound No. 14*).—Hydrogenolysis of *iso*acronycidine in ethanolic solution in the presence of palladium, followed by chromatography on alumina, and repeated crystallization from methanol gave III as colourless needles, m.p. 184–185 °C (Found : C, 61·6 ; H, 6·5 ; N, 4·7%. Calc. for $C_{15}H_{19}O_5N$: C. 61·4 ; H, 6·5 ; N, 4·8%).

(*c*) *Dihydroisoacronycidine* (*(VII) : Compound No. 28*).—Dihydroacronycidine, heated with methyl iodide for 18 hr at 100 °C in a sealed tube, was converted to VII, colourless prisms, m.p. 226·5–228 °C, after several crystallizations from methanol (Found : C, 61·9 ; H, 5·9 ; N, 4·9 ; CH_3O, 31·3 ; $CH_3(N)$, 4·8%. Calc. for $C_{15}H_{17}O_5N$: C, 61·9 ; H, 5·8 ; N, 4·8 ; $3\times CH_3O$, 32·0 ; $CH_3(N)$, 5·2%).

(*d*) *Spectroscopic Technique.*—The spectra of compounds Nos. 6, 7, 8, 21, 22, 23, and 24 were recorded by Dr. R. N. Haszeldine on the double-beam Perkin-Elmer spectrometer at the University Chemical Laboratories, Cambridge, and that of compound No. 11 (as a KCl disk) by Dr. R. G. Gillis of Defence Standards Laboratories, Maribyrnong, Victoria.

The remaining spectra were recorded with either a Perkin-Elmer model 12C spectrometer, converted to double-pass operation, or with a Perkin-Elmer model 21 double-beam spectrophotometer, fitted with a sodium chloride prism. A few of the spectra in the 1450–1700 cm^{-1} region were checked by using a calcium fluoride prism in the single-beam instrument. Solid specimens were measured as mulls in paraffin oil (Nujol), as it is now known that the spectra of many materials obtained from alkali halide pellets undergo changes depending on the nature of the alkali halide and the structure and crystal properties of the substance (see, for instance, Baker 1957 ; Farmer 1957).

Solutions in carbon tetrachloride and chloroform were measured in cells ranging from 0·2–10 mm in length. The solvents used were of " Analar " quality and were dried with silica gel before use. The 2% of ethanol used as a stabilizer in the chloroform was removed just before use by activated alumina.

IV. Acknowledgments

The assistance of Mr. R. Johnstone in the preparation and purification of materials and of Mr. A. Triffett in recording spectra is gratefully acknowledged.

V. References

Baker, A. W. (1957).—*J. Phys. Chem.* **61** : 450.

Barnes, R. B., Gore, R. C., Liddel, U., and Williams, V. Z. (1944).—" Infra-Red Spectroscopy." p. 98. (Reinhold Publishing Corp. : New York.)

Bellamy, L. J. (1958).—" The Infra-Red Spectra of Complex Molecules." 2nd Ed. (Methuen & Co. : London.)

Braunholtz, J. T., Ebsworth, E. A. V., Mann, F. G., and Sheppard, N. (1958).—*J. Chem. Soc.* **1958** : 2780.

Cannon, C. G., and Sutherland, G. B. B. M. (1951).—*Spectrochim. Acta* **4** : 373.

Cook, D. J., Yunghans, R. S., Moore, T. R., and Hoogenboom, B. E. (1957).—*J. Org. Chem.* **22** : 211.

Cooke, R. G., and Haynes, H. F. (1958).—*Aust. J. Chem.* **11** : 225.

Crow, W. D., and Price, J. R. (1949*a*).—*Aust. J. Sci. Res.* A **2** : 255.

Crow, W. D., and Price, J. R. (1949*b*).—*Aust. J. Sci. Res.* A **2** : 282.

Farmer, V. C. (1957).—*Spectrochim. Acta* **8** : 374.

Gibson, J. A., Kynaston, W., and Lindsay, A. S. (1955).—*J. Chem. Soc.* **1955** : 4340.

Goodwin, S., Shoolery, J. N., and Johnson, L. F. (1959).—*J. Amer. Chem. Soc.* **81** : 3065.

Grundon, M. F., and McCorkindale, N. J. (1957).—*J. Chem. Soc.* **1957** : 2177.

Grundon, M. F., McCorkindale, N. J., and Rodger, M. N. (1955).—*J. Chem. Soc.* **1955** : 4284.

Hughes, G. K., and Neill, K. G. (1949).—*Aust. J. Sci. Res.* A **2** : 429.

Johnstone, R., Price, J. R., and Todd, A. R. (1958).—*Aust. J. Chem.* **11** : 562.

LAHEY, F. N., LAMBERTON, J. A., and PRICE, J. R. (1950).—*Aust. J. Sci. Res.* A **3**: 155.
LAMBERTON, J. A., and PRICE, J. R. (1953*a*).—*Aust. J. Chem.* **6**: 66.
LAMBERTON, J. A., and PRICE, J. R. (1953*b*).—*Aust. J. Chem.* **6**: 173.
MASON, S. F. (1957).—*J. Chem. Soc.* **1957**: 4874.
PRICE, J. R. (1959).—*Aust. J. Chem.* **12**: 458.
SHEINKER, Y. N., and POMERANTSEV, Y. I. (1956).—*Zh. Fiz. Khim.* **30**: 79.
WITKOP, B., PATRICK, J. B., and ROSENBLUM, M. (1951).—*J. Amer. Chem. Soc.* **73**: 2641.

THE VIBRATIONAL SPECTRUM OF METHYL *ISO*THIOCYANATE

By A. J. Costoulas* and R. L. Werner*

[*Manuscript received July 2, 1959*]

Summary

Methyl *iso*thiocyanate has been studied in the infra-red region from 10,000 to 250 cm^{-1} as vapour, solid, and melt, while the Raman spectrum has been recorded in the molten condition. The molecule is close to a symmetric top and the bands of the vapour exhibit contours generally similar to that type of molecule.

A satisfactory assignment of all fundamentals has been made. Of interest is the pseudo-symmetric NCS stretching mode which has been assigned to the parallel band at 676 cm^{-1} in the vapour. In the Raman spectrum, the corresponding mode is observed as a strong polarized line at 656 cm^{-1}.

I. Introduction

The most generally accepted configuration of methyl *iso*thiocyanate is one in which the NCS group of the molecule is linear, with the methyl group lying at a slight angle to the axis through those three atoms. No isomerism of the NCS group to SCN has reliably been shown to take place (Goubeau and Gott 1940).

The molecule is, therefore, an asymmetric top with three different moments of inertia, and would have under the most favourable arrangement of atoms a C_s configuration with one plane of symmetry. However, Beard and Dailey (1949) established from an examination of the microwave spectrum of the compound that two of the moments of inertia (I_B and I_C) are close and considerably larger than I_A. Substitution of their results in the formula $s=(2b-a-c)/(a-c)$ of Badger and Zumwalt (1938), where s is the asymmetry number and a, b, and c are equal to $1/I_A$, $1/I_B$, and $1/I_C$, respectively, yields a value of $-0{\cdot}999$ for the asymmetry s, very close to the value of -1 expected for a true symmetric top.

It might well be possible, therefore, to consider the molecule as an accidentally symmetric top possessing higher symmetry, namely, C_{3V}, with the threefold axis through the methyl group lying very close to the top axis of the molecule.

For the case of C_s configuration, a seven-particle system like CH_3NCS should show 15 fundamental frequencies both in scattering and in absorption, 11 of which would belong to class A' and 4 to class A''. The former would be polarized in the Raman effect, while the latter would give depolarized shifts. In the infra-red spectrum, three types of bands in the infra-red spectrum of the vapour would be anticipated depending upon the axis along which the dipole moment changed.

* Department of Physical Chemistry, University of New South Wales, Broadway, Sydney.

Thus, if the change in dipole moment occurs along the minor axis (type *A* band), and if the ratio $\rho = I_A/I_B$ is zero or very small (as here), then the band will appear very similar to a parallel band of a symmetric top (Nielsen 1931) with a strong unresolved central maximum and a series of nearly equidistant lines on either side. If the change in dipole moment takes place along the intermediate axis (type *B* band), then for any value of ρ, a gap with a strong maximum on either side due to *Q* branches should result. For type *C* bands (change of dipole moment along the major axis), a single broad maximum is obtained for the case of small values of ρ.

If, on the other hand, the molecule is considered to possess C_{3v} symmetry on account of its accidental symmetry, then 10 vibrations are expected to occur both in the infra-red spectrum and in the Raman effect. Five should belong to class A_1, and another five to class *E*.

The first class should exhibit a parallel band structure in the infra-red spectrum of the vapour with a *Q* branch small or absent since ρ is very small (Herzberg 1950). The separation of the *P* and *R* branch maxima hould be $11 \cdot 8\ cm^{-1}$ at 25 °C based on the values of the rotational constants obtained by Beard and Dailey (1949) while the separation of the individual lines in the *P* and *R* branches should be $0 \cdot 17\ cm^{-1}$. These bands should, of course, correspond to polarized Raman shifts. The five vibrations of class *E* are expected to give infra-red vapour bands having the broad toothed single maximum characteristic of the perpendicular bands of symmetric top molecules. The separation of the *Q* lines should be $5 \cdot 06\ cm^{-1}$ and a characteristic alternation in intensity of strong, weak, weak, strong would be anticipated.

Since the molecule is only accidentally of a type similar to a symmetric top, it might be expected that some irregularities would appear, for example, in the external modes of the off-axis methyl group.

II. Experimental

(*a*) *Preparation*

The preparation of methyl *iso*thiocyanate was essentially that of Delépine (1908) supplemented by information from Organic Syntheses (Vol. 21, p. 81). The final product was a white solid, melting at 36 °C.

(*b*) *Spectroscopic Studies*

The infra-red spectrum was obtained over the range 10,000–250 cm^{-1} for the vapour as well as molten and solid states while the corresponding Raman spectrum was obtained for the molten material only.

The region from 10,000–2300 cm^{-1} was examined with a Perkin-Elmer Model 112 double-pass spectrometer with LiF prism, while the range 2300–650 cm^{-1} was covered with a double-beam instrument fitted with an NaCl prism constructed by one of us (R.L.W.) as well as with the double-pass instrument. For the region 650–250 cm^{-1}, the double-pass instrument was used with KBr and CsBr prisms.

For the vapour spectrum a spectral slit width of 3 cm^{-1} was obtainable except at the longest wavelengths. For observations on the vapour a gas cell

which could be heated was constructed and was used at temperatures up to 75 °C depending on the band under study.

The Raman spectrum was obtained using a Hilger photoelectric instrument and employing the mercury 4358 Å line for excitation. In order to remove interference from the 4047 Å line of mercury and to keep the sample molten, a saturated solution of sodium nitrite at room temperature was circulated at 60–70 °C about the sample cell. Polarization measurements were made by the method of Cranmer and Werner (1957). For normal studies, a spectral bandwidth of 6 cm^{-1} was used, but this was increased to 8 cm^{-1} for the polarization measurements.

III. Assignment and Discussion

The bands observed in the infra-red spectrum of methyl *iso*thiocyanate are given for the liquid melt and the solid state in Table 1 and for the vapour in Table 2. In Table 3, Raman data for the molten material are given.

Table 1

OBSERVED ABSORPTION BANDS (cm^{-1}) IN THE INFRA-RED SPECTRUM OF METHYL ISOTHIOCYANATE

Solid	Liquid	Solid	Liquid
5175 (v.w)	5190 (v.w)	2438 (w)	2442 (v.w)
5120 (v.w)	5120 (v.w)	2329 (v.w)	
4465 (w)	4460 (w)	2210 (v.s)	2201 (v.s)
4430 (w)	4435 (w)	2129 (v.s)	2124 (v.s)
4365 (v.w)	4370 (w)	2041 (ssh.)	2030 (ssh.)
3310 (v.w)	3301 (v.v.w)	1723 (v.w)	
3169 (v.v.w)		1636 (v.wbr.)	
3082 (w)	3082 (v.w)	1541 (wbr.)	1538 (wbr.)
2993 (w)	3004 (w)	1443 (m)	1444 (msh.)
2931 (s)	2911 (s)	1408 (s)	1410 (s)
2861 (m-s)	2863 (m)	1220 (w)	1226 (w-m)
2840 (s)	2841 (m-s)	1086 (m)	1083 (m)
2793 (m)	2796 (m)	1005 (v.wbr.)	1012 (v.w)
2763 (m)	2765 (m)	884 (w)	886 (w)
2610 (w)		645 (s)	647 (s)
2572 (w)		576 (m-s)	567 (m)
2547 (w)	2549 (w)	449 (m)	453 (m)
2511 (w)	2517 (w)	353 (v.w)	353 (v.w)

Before discussing assignments in detail, it is relevant to enquire as to the general nature of the spectra produced. Of particular interest is the infra-red spectrum of the vapour which shows mainly two types of bands, one resembling closely the parallel bands of symmetric top molecules while the other is similar to the perpendicular bands of such molecules. As is common, the separation of the Q branches in the perpendicular bands varies, in this case from 5 to 9·5 cm^{-1}, but it is constant for any one band. The fine structure of the band near 3000 cm^{-1} shows clearly the interesting strong, weak, weak, strong alternation in intensity which is characteristic of symmetric tops.

TABLE 2

OBSERVED ABSORPTION BANDS IN THE INFRA-RED SPECTRUM OF METHYL ISOTHIOCYANATE IN THE VAPOUR STATE

Frequency and Intensity (cm^{-1})	Type	Assignment	Frequency and Intensity (cm^{-1})	Type	Assignment
4525 (v.w)		2×2223	2069 (v.v.ssh.)		
4370 (v.wsh.)			1474 (m)	Perpendicular	Asymmetric CH_3 deformation
4225 (v.w)		4×1090			
4140 (v.wsh.)			1426 (v.s)	Parallel	Symmetric CH_3 deformation
2970 ? (s)	Perpendicular	Asymmetric CH_3 stretch	1272 (v.w)		903+353
2952 (s)	Parallel	Symmetric CH_3 stretch	1176 (m)	Perpendicular ?	Out-of-plane CH_3 rock
2900 (m)	Parallel	2223+676	1090 (m-s)	Hybrid	CH_3–N stretch
2820 (w)	Parallel	2×1426	796 (v.v.w)		433+353
2781 (w-msh.)	Parallel	$1426+2\times676$	676 (s)	Parallel	Symmetric NCS stretch
2762 (m)	Parallel	2088+676			
2581 (v.w)	Parallel	1426+1090	532 (s)	Perpendicular	Out-of-plane skeletal bend
2479 (v.v.w)		2970—532			
2246 (msh.)		2952—676	502 (msh.)		
2223 (v.v.s)	Parallel	2×1090	482 (msh.)		
2108 (v.ssh.)		1426+676	433 (s)	Perpendicular	In-plane skeletal bend
2088 (v.v.s)	Parallel	Asymmetric NCS stretch	353 (v.w)	Perpendicular ?	CH_3–N bend

TABLE 3

THE RAMAN SPECTRUM OF METHYL ISOTHIOCYANATE IN THE MOLTEN STATE

Raman Shift	Scatter Coefficient*	ρ_n	Assignment
2989	0·44	0·86	Asymmetric CH_3 stretch
2936	2·88	0·21	Symmetric CH_3 stretch
2671	0·22	0·62	
2208	0·38	0·74	2×1097
2118	0·36	0·76	Asymmetric NCS stretch
1885 ?	0·02	0·64	2989—1097
1767 ?	0·02	0·39	1418+353
1418	0·71	0·75	Symmetric CH_3 bend
1361 ?	0·03	0·65	
1097	0·28	0·15	CH_3–N stretch
903	0·08	0·33	In-plane CH_3 rock
656	0·53	0·33	Symmetric NCS stretch

* The scattering coefficient was measured relative to the carbon tetrachloride 458 cm^{-1} line, corrected for the sensitivity curve of the detector.

In the Raman spectrum of the melt, eight polarized lines were found. Of these, it will be shown that 2208 cm^{-1} is not a fundamental and two others are so very weak that the polarization information may be in error. Therefore, only five certain fundamentals of this type remain, which is suggestive of C_{3v} symmetry. The combination of evidence above is quite typical of a symmetric top molecule and suggests that methyl *iso*thiocyanate may be interpreted as a molecule of this type.

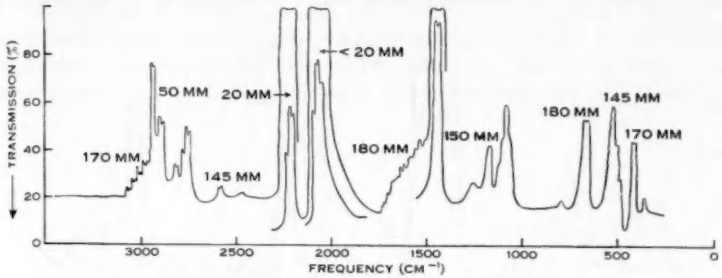


Fig. 1.—Infra-red spectrum of methyl *iso*thiocyanate (vapour).

On the other hand, the band at 1090 cm^{-1} in the infra-red spectrum of the vapour, resembles neither a parallel nor a perpendicular band and, in fact, is similar to a type *C* band of an asymmetric top, in which the change in dipole moment takes place along the major axis of the molecule. Considered overall, it does not seem unreasonable to assume that the molecule is an approximation to a symmetric top and in what follows, this assumption has been adopted to facilitate the assignment of the bands.

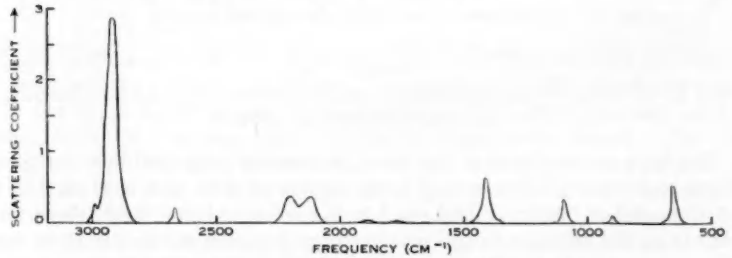


Fig. 2.—Raman spectrum of methyl *iso*thiocyanate (melt).

The vapour spectrum of methyl *iso*thiocyanate from 3500 to 250 cm^{-1} is reproduced in Figure 1 while the Raman spectrum is shown in Figure 2. In Figure 4, the fine structure of the bands in the region of the CH stretching frequencies is shown in detail. The strong parallel band centred at 2952 cm^{-1} which has *P* and *R* branch maxima at 2946 and 2958 cm^{-1} respectively, is clearly to be assigned to the symmetric CH stretching frequency. In the Raman spectrum, the corresponding line is at 2936 cm^{-1} and is strongly polarized. The

drop of 16 cm^{-1} from vapour to melt is not unexpected. On the high frequency side of this band can be seen part of a perpendicular band exhibiting the characteristic fine structure referred to earlier. The separation between sub-bands in this case is 9·5 cm^{-1} indicative of strong Coriolis coupling. It is difficult to determine the origin of this perpendicular band which probably lies about 2970 cm^{-1}. In the Raman spectrum of the melt, the depolarized and rather weak line at 2989 cm^{-1} corresponds to the vapour band and they are assigned to the asymmetric CH stretching mode which is degenerate in symmetric top molecules.

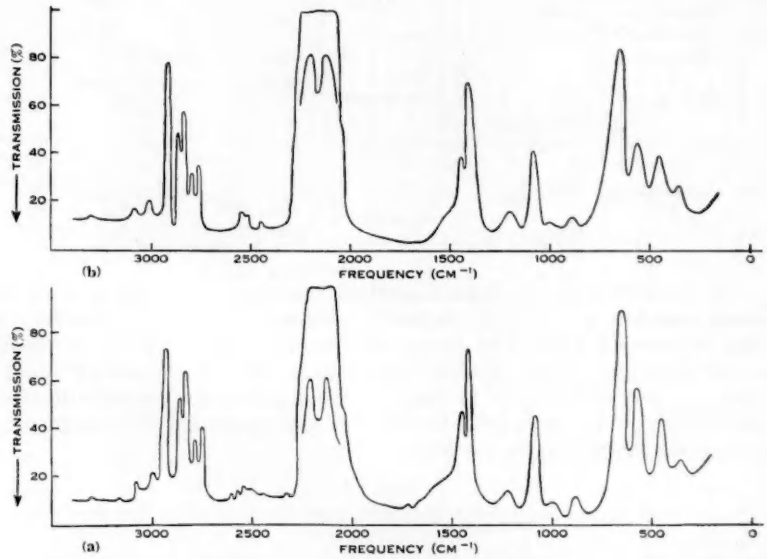


Fig. 3.—(*a*) Infra-red spectrum of methyl *iso*thiocyanate (melt); (*b*) infra-red spectrum of methyl *iso*thiocyanate (solid).

The infra-red spectrum of the solid and molten compound in this region is difficult to interpret. Two strong bands appear at 2931 and 2840 cm^{-1} in the solid state and at 2911 and 2841 cm^{-1} in the melt. At first sight, these would appear to be the asymmetric and symmetric modes corresponding to those found in the vapour and in the Raman effect. However, it is difficult to account for the large discrepancy between the sets of observations, particularly between the Raman and infra-red results for the molten compound for which the state and temperature were the same. All results were carefully rechecked and the difference does not appear to be experimental error.

It is believed that the infra-red spectrum of the vapour and the Raman results are of greater use since:

(i) Overtone and combination bands are notably weaker in the Raman effect, thus facilitating the choice of fundamentals;

(ii) polarization data and relative intensities of the CH bands in the Raman effect conform to the usual appearance of methyl modes;

(iii) the parallel and perpendicular bands of the vapour spectrum are in reasonable agreement with the Raman shifts.

It may well be that the appearance of the infra-red spectrum in the condensed state has been affected by overtone and combination bands of enhanced intensity which have appeared in the solid and molten conditions.

In the spectral interval between 2350 and 2000 cm^{-1}, there is found a very strong and broad pair of bands. Similar bands are found in the spectra of *iso*cyanates, *iso*thiocyanates, and HNCO (Herzberg and Reid 1950). In the infra-red spectrum of the vapour it can be clearly seen that both bands have a

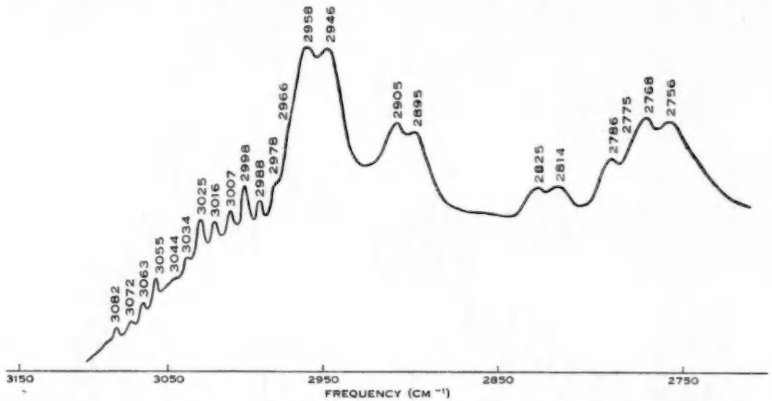


Fig. 4.—Vapour spectrum of methyl *iso*thiocyanate in the CH region.

parallel type band structure. The lower frequency band centred at 2088 cm^{-1} (branch maxima at 2082 and 2093 cm^{-1}) is assigned to the pseudo-asymmetric NCS stretching mode because of its intensity and proximity to 1963 cm^{-1}, the value found for a similar mode in HNCS (Reid 1950*a*, 1950*b*). The higher frequency band, which is rather weaker in the vapour is centred at 2223 cm^{-1} (branch maxima at 2218 and 2228 cm^{-1}). This is assigned to the first overtone of the band at 1090 cm^{-1}. This latter is assigned to the CH_3—N stretching mode and would be of the same symmetry class as the pseudo-asymmetric NCS stretching mode. Fermi resonance is likely to occur, raising the overtone from approximately 2180 to 2223 cm^{-1}. At the same time the fundamental has been depressed in frequency.

It seems probable that the shoulder on the high frequency side of the fundamental at 2108 cm^{-1} is to be assigned to the combination 1426+676 cm^{-1} (=2102 cm^{-1}) but the small band on the low frequency side at 2069 cm^{-1} could not be assigned. However, it is possible that perpendicular components are involved in both cases. The assignment of the condensed state follows that of the vapour in an obvious manner.

In the region 1600–1300 cm^{-1}, a strong parallel band is observed in the infra-red spectrum of the vapour with perpendicular-band structure on its high frequency side (Fig. 5). The parallel transition is centred at 1426 cm^{-1} with P and R branch maxima at 1420 and 1432 cm^{-1}, and is assigned to the symmetrical CH_3 deformation frequency. The spacing of the lines in the perpendicular component, as well as their intensity variation, is irregular, and this may be partly accounted for by insufficient resolving power. However, another factor which may have to be taken into consideration is that since the molecule is only an accidentally symmetric top, the parallel transition may show some perpendicular component, which because of the closeness of the doubly degenerate mode nearby, will be superimposed on the perpendicular band, and since the separation between the lines of the sub-bands in the two cases will not be the same,

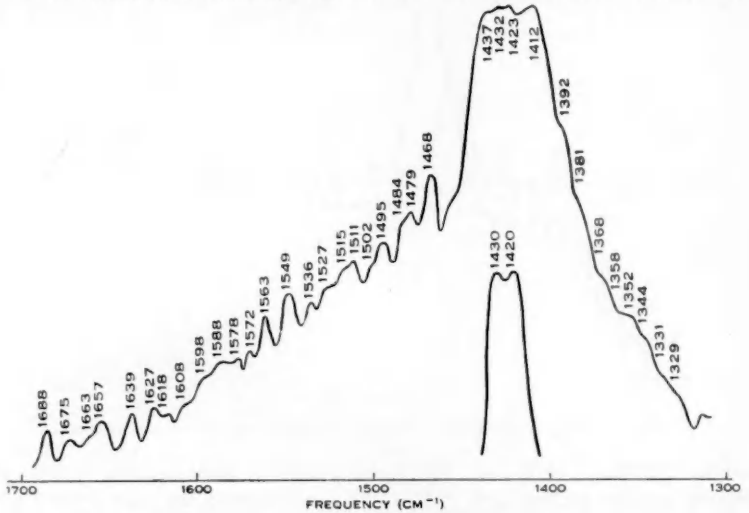


Fig. 5.—Methyl deformation modes of methyl *iso*thiocyanate in the vapour.

the bands will not coincide, and the structure will be very irregular. The centre of the perpendicular band (asymmetric CH_3 deformation frequency) is placed between the two strongest lines at 1479 and 1468 cm^{-1}, that is, at 1474 cm^{-1}.

The Raman spectrum exhibits a strong, polarized shift at 1418 cm^{-1} which is assigned to the symmetric CH_3 deformation mode, but it does not show a depolarized line nearby, which could be attributed to the asymmetric deformation vibration. The infra-red spectrum of the solid and liquid showed strong absorptions at 1408, 1445 cm^{-1} and 1410, 1444 cm^{-1} respectively.

The former band in both cases has twice the intensity of the latter, and is assigned to the symmetric CH deformation frequency, while the other is attributed to the asymmetric mode.

The weak centre appearing at 1272 cm^{-1} in the vapour spectrum can conveniently be assigned as the sum of 903 and 353 giving 1256, an anharmonicity

difference of 16 cm^{-1} which is quite reasonable. Its structure could not be followed because of noise and the assignment could not therefore be checked in this way.

The methyl rocking modes are known to occur in the region 1250–800 cm^{-1}. By analogy with CH_3NCO (Eyster and Gillette 1940), dimethyl acetylene (Crawford, 1939), and other molecules, the medium intensity absorption band observed at 1176 cm^{-1} in the vapour spectrum is assigned to the out-of-plane CH_3 rocking motion, while the weak Raman shift at 903 cm^{-1} is attributed to the corresponding in-plane mode. The weak centre at 796 cm^{-1} is then assigned to the sum 433 + 353 cm^{-1}.

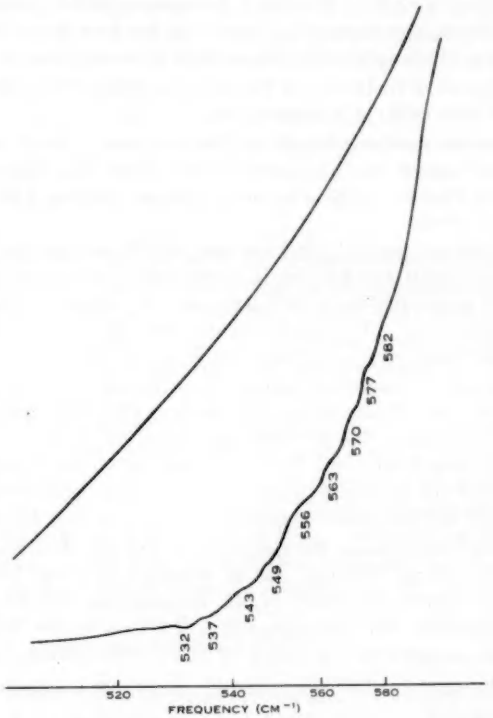


Fig. 6.—Out-of-plane skeletal bending mode of methyl *iso*thiocyanate (vapour spectrum).

In the infra-red spectrum of the solid and melt, three weak bands are to be found in the range 1230–800 cm^{-1}. Two of these, 1226, 886 cm^{-1} in the melt and 1220, 884 cm^{-1} in the solid, are assigned to the out-of-plane and in-plane CH_3 rocking modes respectively, while the centre at 1012 cm^{-1} (melt) and 1005 cm^{-1} (solid), whose intensity is lower, is thought to be due to the sum 567 + 453 cm^{-1} and 576 + 449 cm^{-1} respectively.

It should be emphasized that since the molecule is only accidentally a symmetric top, the degenerate methyl rocking vibrations, being external modes, need not follow the behaviour of the degenerate CH stretching vibrations, which are internal modes. They could, therefore, split up, even though this does not appear to happen with the stretching frequencies.

A fairly strong band showing pronounced hybrid character is observed at 1090 cm^{-1} in the infra-red spectrum of the vapour. In the Raman record, a fairly strong, polarized shift at 1097 cm^{-1} has been found. This would indicate that the transition is of class A_1 for symmetric top configuration, or of class A' for point group C_s.

It is assigned to the CH_3-N stretching frequency, and because of the position of the methyl group, the change in dipole moment does not take place exactly along the top axis of the rotator but at an angle to it, resulting in a hybrid band.

The corresponding frequency in the infra-red spectra of the solid and melt occurs at 1086 and 1083 cm^{-1} respectively.

The assignment is strengthened by the fact that in methylamine, Cleaves and Plyler (1939) assign the C—N stretching mode to a band at 1045 cm^{-1}, and Borden and Barker (1938) attribute 1034 cm^{-1} to the CH_3-O stretching mode in methyl alcohol.

The fairly strong, parallel band centred at 676 cm^{-1} in the vapour with P and R branches at 670 and 681 cm^{-1}, respectively, is assigned to the pseudo-symmetric NCS stretching mode and its polarized nature in the Raman effect supports the assignment. The band is much lower in frequency than the C=S vibration in other molecules (e.g. 1121 cm^{-1} in thiophosgene, Thompson 1938).

By comparing the force constants and frequencies of the CS group in CH_3NCS and $CSCl_2$ with those of the CO group in CH_3–NCO and $COCl_2$, a value of 738 cm^{-1} is obtained for the CS stretching vibration in CH_3NCS. If the force constants in the ions $NCSe^-$ and NCS^- are assumed to be the same, and if the different masses of the Se and S atoms are taken into consideration, a value of 630 cm^{-1} is found for the pseudo-symmetric NCS stretching frequency.

An effect that would lower the frequency of the CS vibration in this type of compound is the strong coupling that is expected to occur between the two adjacent double bonds, resulting in an increase of the frequency of the antisymmetric motion, and a corresponding decrease of the frequency of the symmetric mode, almost to the range of the C—S stretching vibration. In addition, contributions by the structure $CH_3-\overset{+}{N}\equiv C-\overset{-}{S}$ would lower the pseudo-symmetric mode.

The corresponding vibration in the infra-red spectrum of the solid is found at 645 cm^{-1}, and of the melt at 647 cm^{-1}.

The skeletal deformation modes are expected to appear in the region below 600 cm^{-1}. Unfortunately, the Raman record did not show any bands in this region, but in the infra-red spectrum three distinct centres were observed. In the vapour, they were found at 532, 433, and 353 cm^{-1}, in the solid at 576, 449, and 353 cm^{-1}, and in the melt at 567, 453, and 353 cm^{-1} respectively, with the bands in the solid being stronger and slightly sharper than those in the melt.

The 532 cm^{-1} transition in the infra-red spectrum of the vapour had the structure of a pure perpendicular band on its high-frequency side, with two or three shoulders on the low-frequency side, Figure 6. The average separation of the sub-bands is 6·25 cm^{-1}, indicating a certain amount of Coriolis interaction.

By analogy with CH_3NCO (Eyster and Gillette 1940), HNCO (Herzberg and Reid 1950), HNCS (Reid 1950*a*, 1950*b*), and other molecules (Crawford 1939), and on the basis of its perpendicular contour, the band is assigned to the out-of-plane skeletal bending vibration of the CH_3NCS molecule.

The fairly strong centre at 433 cm^{-1} is a hybrid band with an extra two peaks on both sides of the P and R branches shown. The separation between the sub-bands is found to be approximately 4 cm^{-1}, and a strong parallel component is indicated. This fixes it as the skeletal in-plane deformation mode, since it has been found by other workers that this vibration is less perpendicular in character than the out-of-plane mode.

The last band observed in the infra-red spectrum at 353 cm^{-1} is very weak, and its contour could not be followed in the vapour because of noise. It is assigned to the $CH_3—NC$ angle deformation, and not to the methyl torsional vibration because the latter would occur at a much lower frequency (Hadni 1954*a*, 1954*b*).

It will be noticed that in both the perpendicular skeletal modes just discussed, the frequency of the vibration in the vapour is lower than that observed in the solid and melt. This is probably due to some form of dipolar interaction taking place between the molecules of the condensed phases, which is known to lower the frequencies of stretching vibrations, and to increase those of the skeletal deformation modes.

The region above 2400 cm^{-1} in the infra-red spectra of the solid, melt, and vapour contains a number of overtone and combination bands which are listed in Tables 1 and 2, together with the most likely assignments in the case of the vapour. This state only has been assigned, because the contours of the bands allowed a check to be made in a number of cases. Further, the absence of intermolecular interaction would tend to cause less irregularity in the selection rules. It will be observed that most of these are combinations or overtones of bands of class A_1 on the symmetric top model. The resultant bands are also of this class and exhibit typical parallel band structure.

IV. Thermodynamic Functions for Methyl *iso*Thiocyanate

The thermodynamic functions tabulated for methyl *iso*thiocyanate are the free-energy function—$(F^0-H_0^0)/T$, the heat content function $(H^0-H_0^0)/T$, and the heat capacity (C_p^0). Standard equations, given by Janz (1955), have been used for the calculation of the translational and rotational contributions to the functions mentioned. For the evaluation of the vibrational contributions the tables of Torkington (1950) have been consulted. All the fundamentals are available with the exception of the methyl torsional mode, for which a value of 270 cm^{-1} was chosen. This is the value found experimentally by Hadni (1954*a*, 1954*b*) for the methyl torsional mode in dimethyl ether.

Substitution of this value in the formula given by Herzberg (op. cit., p. 226), namely,

$$W_t = n\sqrt{(V_0 A_1 A_2 / A)}\ \text{cm}^{-1}$$

gives the height of the barrier restricting rotation about the C—N bond. Here A_1 and A_2 are the rotational constants corresponding to the moments of inertia of the two parts of the molecule carrying out the torsional motion and A is the rotational constant corresponding to the sum of the two moments above. In this case a value of 1641 cm^{-1} for V_0 was obtained corresponding to a barrier of 4700 cal/mole. The thermodynamic functions are summarized in Table 4.

TABLE 4

THERMODYNAMIC FUNCTIONS

R, *Tr*, and *V* are the rotational, translational, and vibrational contributions respectively

Absolute Temperature (°K)	$-(F^0-H_0^0)/T$		$(H^0-H_0^0)/T$		C_p^0		S^0	
	R+*Tr*	*V*	*R*+*Tr*	*V*	*R*+*Tr*	*V*	*R*+*Tr*	*V*
273·15	54·12	1·33	5·96	2·84	7·95	7·25	62·07	4·17
298·15	54·81	1·59	5·96	3·25	7·95	8·02	62·76	4·84
300	54·85	1·61	5·96	3·28	7·95	8·08	62·80	4·89
400	57·15	2·77	5·96	4·84	7·95	10·91	65·10	7·64
600	60·37	5·28	5·96	7·65	7·95	15·41	68·32	12·93

Examination of Torkington's (loc. cit.) tables shows that an alternative choice of frequency for the torsional mode has only a small effect. For example, if this frequency were chosen as low as 200 cm^{-1} the increments at 298·15 °K would only be as follows : $-(F^0-H_0^0)/T$ +0·32 ; $(H^0-H_0^0)/T$ +0·21 ; C_p^0 +0·11.

V. REFERENCES

BADGER, R. M., and ZUMWALT, L. R. (1938).—*J. Chem. Phys.* **6**: 711.

BEARD, C. I., and DAILEY, B. P. (1949).—*J. Amer. Chem. Soc.* **71**: 929.

BORDEN, A., and BARKER, E. F. (1938).—*J. Chem. Phys.* **6**: 553.

CLEAVES, A. P., and PLYLER, E. K. (1939).—*J. Chem. Phys.* **7**: 563.

CRANMER, V., and WERNER, R. L. (1957).—*Aust. J. Chem.* **10**: 87.

CRAWFORD, B. L., JR. (1939).—*J. Chem. Phys.* **7**: 140.

DELÉPINE, M. (1908).—*Bull. Soc. Chim. Paris* **3**: 641.

EYSTER, E. H., and GILLETTE, R. H. (1940).—*J. Chem. Phys.* **8**: 369.

GOUBEAU, J., and GOTT, O. (1940).—*Ber. dtsch. chem. Ges.* B **73**: 127.

HADNI, A. (1954*a*).—*C.R. Acad. Sci. Paris* **238**: 573.

HADNI, A. (1954*b*).—*C.R. Acad. Sci. Paris* **239**: 349.

HERZBERG, G. (1950).—"Infrared and Raman Spectra of Polyatomic Molecules." p. 421. (van Nostrand Co. Inc.: New York.)

HERZBERG, G., and REID, C. (1950).—*Disc. Faraday Soc.* **9**: 92.

JANZ, G. J. (1955).—*Quart. Rev. Lond.* **9**: 229.

NIELSEN, H. H. (1931).—*Phys. Rev.* **38**: 1432.

REID, C. (1950*a*).—*J. Chem. Phys.* **18**: 1512.

REID, C. (1950*b*).—*J. Chem. Phys.* **18**: 1544.

THOMPSON, H. W. (1938).—*J. Chem. Phys.* **6**: 748.

TORKINGTON, P. (1950).—*J. Chem. Phys.* **18**: 1373.

THE CONVERSION OF OXYGEN IN COMPOUNDS TO WATER FOR ISOTOPIC ANALYSIS BY A DENSITY TECHNIQUE

By I. LAUDER* and I. R. WILSON*

[*Manuscript received April* 28, 1959]

Summary

A method is described which enables oxygen in numerous compounds to be isolated in the form of water as a preliminary step to the determination of the isotopic composition of the oxygen by a density technique. The oxygen in the compound is converted to carbon monoxide or dioxide. These gases are reduced by hydrogen in the presence of a pure nickel catalyst to yield water and hydrocarbons. A small memory effect is shown, but this is eliminated by the first sample put through the process. The process is carried out on a scale such that 1 mmole of water is produced for each reduction. The time involved is about 2 hr per sample.

I. INTRODUCTION

Numerous oxygen-18 tracer experiments have been followed by isolating the oxygen in the compound under investigation in the form of water, the density of which is then determined by some suitable technique. No general method for this type of analytical procedure has been described in the literature. A fundamental requirement of any method is that mixing of the oxygen in the compound with extraneous oxygen, in the free or combined state, must be reduced to a minimum and the effect eliminated completely by repetition or continuation of the procedure.

The ter Meulen (1922) method of conversion of oxygen in compounds to water as modified by Russell and Fulton (1933) has been used by Bunton and Wood (1955) for the isotopic analysis of oxygen in *tert.*-butanol. These authors comment, " dilution and memory effects are large ; therefore considerable time has to elapse between samples, and large samples (*c.* 2 g) of the alcohol are desirable ". Anbar *et al.* (1955) have also investigated the method and report, " the procedure is time-consuming and requires relatively large amounts of material, particularly since, in order to minimize ' memory ' effects, several samples have to be processed before a final analysis is made ". A procedure has been developed by Kudryavtsev, Ottesen, and Kursanov (1956) in which the organic compound is subjected to exhaustive hydrogenation over a nickel catalyst. The catalyst is previously equilibrated with respect to oxygen-18 by a fourfold passage of oxygen-18 water over it.

A slightly different procedure was introduced by Herbert and Lauder (1938). The organic compound was decomposed in the vapour state by contact with a white-hot platinum filament. The oxides of carbon and other gases formed

* Department of Chemistry, University of Queensland, Brisbane.

were then passed into a reduction system and excess hydrogen was added. In the presence of a pure nickel catalyst, carbon monoxide and carbon dioxide were reduced to methane and water. No account of memory effects associated with the technique is given in the original paper. The procedure has now been investigated more fully and results are presented below.

II. Experimental

(i) *Nickel Catalyst.*—The nickel catalyst is prepared by the thermal decomposition of nickel formate prepared as described by Bircumshaw and Edwards (1950) or by the decomposition of nickel oxalate prepared as described by Allen and Scaife (1954). The nickel salt is decomposed *in situ* in the catalyst vessel (see Fig. 1). Sufficient compound to give 2 g of nickel is introduced through the end *A*. During the thermal decomposition small solid particles tend to be carried off with the gas. To avoid contamination of the reduction system a temporary vacuum line is sealed on at *A*. The U-bend in this line is packed with glass-wool. The mercury seal stopcocks 1 and 2 are turned to the off-position and the catalyst vessel is evacuated. Other sections of the system are also evacuated.

The most active catalysts are obtained by decomposition of the thoroughly dried nickel compound at as low a temperature as possible. The temperature of the catalyst vessel is raised gradually over a period of 2–3 hr to 180–190 °C and maintained at this level for 8 hr with continuous pumping to ensure complete removal of water of crystallization and adsorbed water from the salt. From time to time the U-bend in the temporary vacuum line is cooled to −80 °C to check whether water is still coming off. The decomposition of the nickel formate is brought about by heating at 210–260 °C and in the case of the oxalate at 290–320 °C. The time required is about 4 hr. The end of the process is indicated by the pressure falling to about 10^{-4} mm Hg. The temporary vacuum line is sealed off at *A*. Hydrogen is added to the reduction system to a pressure of 20–30 cm Hg and the trap *B* is cooled to −80 °C. Circulation of the hydrogen over the nickel catalyst is brought about by a thermal syphon caused by heating 12 in. of vertical tubing to 400 °C, as shown in Figure 1. The catalyst is maintained at 300–320 °C. A small quantity of water is formed and this is frozen out in the trap *B*. After 2–3 days no further significant amount of water is formed. The catalyst is then ready for use. The time required for the catalyst to reach a state where it will give up no further amount of oxygen is shortened by pumping out the hydrogen periodically and then adding fresh hydrogen. In all transfer or evacuation procedures taps 1 and 2 are turned off to prevent water vapour from the trap *B* getting back into the catalyst vessel.

It is most important to keep air out of the reduction system during the life of the catalyst. If air gains access it is necessary to reduce the catalyst once again but the original activity may never be recovered depending on the amount of oxygen uptake by the catalyst. A good catalyst will reduce quantitatively a millimole of carbon monoxide in about 2 hr, whereas a catalyst which has been affected by air may take 10 times as long for the same reduction. No difference in catalytic activity was observed between catalysts prepared from nickel formate and those prepared from nickel oxalate.

When the catalysts are not in use, the complete system is filled to 1 atm pressure with pure dry hydrogen. The mercury in the manometer attached to the reduction system is covered with " Apiezon oil " and the stopcock to the manometer is normally in the off-position. This manometer is not pumped while the stopcocks 1 and 2 to the catalyst section are open. All the essential stopcocks, 1 to 6, are of the mercury seal type. When it is necessary to regrease stopcocks 1 and 2, one at a time is removed and a stream of pure dry hydrogen is maintained through the catalyst vessel to prevent access of air. " Silicone " grease is used. All reductions are carried out with the nickel catalyst heated to 270 °C and a pressure of hydrogen 20–40 cm Hg is used.

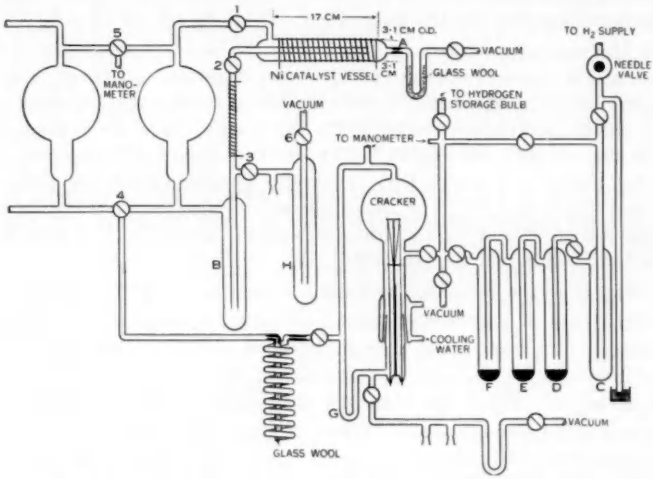


Fig. 1.—The reduction, cracking, and hydrogen purification systems.

(ii) *Hydrogen.*—Electrolytic cylinder hydrogen is purified by passage through trap *C* cooled to −80 °C and then through three traps *D*, *E*, *F* connected in series. Each of the latter contains 5 g of sodium metal and is heated electrically to 300 °C. The dimensions of the traps are the same as those shown for the nickel catalyst vessel. The flow rate is adjusted to 0·5 l/hr by means of a needle valve.

(iii) *Method.*—For compounds which are readily volatile and which give good yields of carbon monoxide or carbon dioxide on thermal decomposition, for example, acetic acid, ether, acetaldehyde, methanol, and ethanol, the type of pyrolysis or cracking apparatus, illustrated in Figure 1, is used. The volume of the cracker is large enough to hold sufficient of the compound to yield 1 mmole of water from the reduction system without the saturation vapour pressure of the compound being exceeded in the cracker under working conditions. For all compounds mentioned above except ethanol, a minimum volume of about 400 ml is satisfactory. For ethanol, a larger volume is necessary as during pyrolysis

only two-thirds of the oxygen in the ethanol is converted to oxides of carbon, the remainder appearing as water.

Decomposition is brought about by circulation of the organic compound in the vapour state over a white-hot platinum wire 0·008 in. diameter by 15–18 in. long. This wire is supported on light quartz rods after the style of an old-fashioned electric light bulb. The reaction appears to be complete in 10–15 min but circulation of the gases over the filament is allowed to continue for 1 hr to minimize the possible transfer of small amounts of organic matter to the reduction system. At the end of this period, the U-bend *G* is cooled to −80 °C and the gases are allowed to circulate for another 5 min. Most of the condensable material is frozen out in this U-bend before the gases are transferred to the reduction system via the long spiral coil (12 ft) cooled to −80 °C.

With the present set-up the volume of the reduction system is about eight times that of the cracking system and the gases from the cracker are allowed to flow directly into the reduction system. The hydrogen gas required for the reduction, 20–40 cm Hg, is introduced via the cracker, thus achieving a slightly better transfer of gas. The gases in the cracker could be transferred quantitatively by means of a manual or automatic Toepler (Urry and Urry 1956; Roberts and Madison 1957). A more general method of isolating oxygen in compounds quantitatively in the form of carbon monoxide in a pure state is described by Lauder and Zerner (1959).

The trap *B* in the reduction system is cooled to −80 °C; the current for the thermal siphon is turned on; taps 1 and 2 are opened, the temperature of the catalyst having been previously adjusted to 270 °C. The progress of the reduction is followed by observing the changes in pressure on the manometer attached to tap 5. When the reduction is finished, tap 2 is closed, and the system is evacuated through tap 3. When the pressure has fallen to 10^{-3} mm Hg tap 1 is closed, trap *H* cooled to −80 °C, the carbon dioxide–acetone bath is removed from trap *B*, and the system is pumped to transfer the water from *B* to *H*. Tap 3 is then closed. When the pressure is 10^{-4} mm Hg or lower, the water is transferred from *H* by sublimation from 0 to −80 °C to a suitable vessel—a tap vessel—attached to the B14 ground-glass cone. A small piece of pure gold metal 10 by 2 by 0·5 mm is always placed in a tap vessel in which water for density measurement is stored. The gold takes up any traces of mercury which may have condensed in the vessel during the transfer. Tap vessels containing samples of water are stored in a desiccator to prevent contamination by atmospheric moisture should the vessels leak. Water samples are purified by the technique described by Lauder and Wilson (1959) before measurement of density.

The reduction system including catalyst section is pumped before the next sample is introduced. If samples of the same material are being processed, pumping for 10 min is adequate; if samples of different isotopic composition are going through, the system should be pumped for half an hour. As one sample is always processed to remove memory effects, long pumping of the catalyst is not essential.

III. Results and Discussion

Typical graphs showing rates of cracking and reduction are given in Figures 2 and 3 respectively. These are self-explanatory.

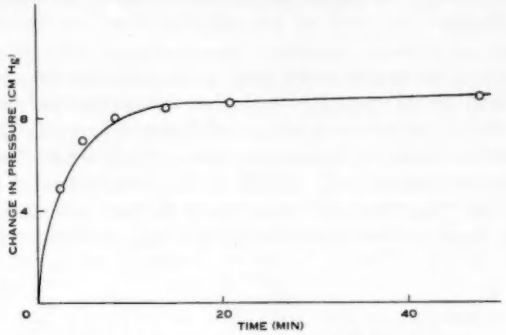


Fig. 2.—Graph howing rate of pyrolysis of ethanol.

(i) *Memory Effects.*—Some results illustrating the memory effect of the technique are given in Table 1. Densities are determined by the modified

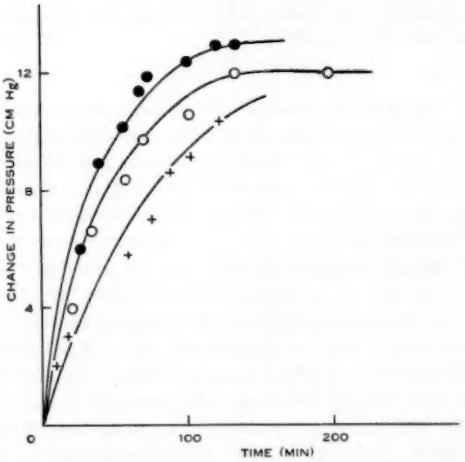


Fig. 3.—Graphs showing rate of reduction of carbon monoxide from 1 mmole of ethanol.

● Pressure of hydrogen, 45·4 cm Hg; catalyst temperature 270 °C.
○ Pressure of hydrogen, 28·6 cm Hg; catalyst temperature 250 °C.
+ Pressure of hydrogen, 8·3 cm Hg; catalyst temperature 210 °C.

Gilfillan-Polanyi micropyknometer method as described by Lauder (1959). The first result shown in Table 1 is for a sample of methanol of normal isotopic composition put through the techniques immediately after preparation of a

catalyst. Water of normal density within experimental error was obtained. The second and third results were obtained for successive samples of the same oxygen-18 methanol. Then two samples of normal methanol were treated, followed by another pair of duplicate samples of oxygen-18 methanol different from the first supply.

The differences between duplicate measurements, 22, 23, and 18 p.p.m. respectively, suggest the presence of a more or less constant amount of exchangeable oxygen either in the cracking, reduction, or purification technique. The only parts of the cracking vessel where exchange of oxygen might occur are the light quartz rods used to suspend the filament. To check the possibility of a memory effect at these points a sample of water, actually the first sample of the series (5 p.p.m.), was circulated over the white-hot platinum filament in the cracking vessel after the last heavy sample (377 p.p.m.) had been put through.

TABLE 1

RESULTS ILLUSTRATING MEMORY EFFECTS OF THE CATALYST

Sample	Excess Density (p.p.m.)	Sample	Excess Density (p.p.m.)
1. Normal methanol ..	5	5. Normal methanol ..	2
2. Oxygen-18 methanol	227	6. Oxygen-18 methanol	359
3. Oxygen-18 methanol	249	7. Oxygen-18 methanol	377
4. Normal methanol ..	25		

After purification the excess density was found to be 2 p.p.m. This result showed that no appreciable exchange of oxygen could occur between the silica and the gases circulating in the cracker. Lauder and Wilson (1959) found a slow exchange of oxygen between water and silica at 750 °C, while Brandner and Urey (1945) found that exchange between silica and oxides of carbon does not occur at appreciable rates till much higher temperatures are reached.

The memory effect in the technique used for purifying the water samples before density measurement is known (Lauder and Wilson 1959). At the temperature used for the purification of the samples of water referred to here, namely 400 °C, no measurable exchange of oxygen between silica and water was detected under the experimental conditions used. Hence the memory effect observed must be in the catalyst section. To minimize it, two reduction systems were joined to the one cracking system. One reduction system was kept for samples of normal isotopic composition, the other system for heavy samples.

When a number of samples are to be processed, they are arranged if possible in order of increasing or decreasing isotopic content depending on the state of the catalyst, after its last use. In this way memory effects are greatly reduced and for the work reported here one sample is usually sufficient to eliminate the memory. In Table 2 are shown results obtained from a series of consecutive crackings and reductions. Before sample No. 1 was processed, a sample of oxygen-18 alcohol was put through the cracking and reduction procedure to "bring up" the catalyst because it had been in contact with oxygen of normal

isotopic composition. The results refer to two different samples of oxygen-18 methanol treated in triplicate. The excess densities of samples Nos. 3 and 6 were not measured because of the agreement between the preceding results. Therefore, it is possible to conclude that the memory effect has been reduced to a very small level and small samples can be processed contrary to what has generally been claimed.

(ii) *Surface Area of Catalysts.*—A study of the surface areas of nickel catalysts prepared under different conditions from nickel formate was made to assist in selecting the best preparative conditions. Surface areas were determined by the usual B.E.T. method (Brunauer, Emmett, and Teller 1938) using argon as the absorbate and a temperature of −183 °C. The surface area was found to diminish the longer the nickel was maintained at the temperature used for decomposing the formate. For decomposition times of 12, 21, and 38 hr at 200 °C, the surface areas found were 8·8, 7·0, and 5·9 $m^2 g^{-1}$ respectively.

TABLE 2

RESULTS SHOWING THE ELIMINATION OF MEMORY EFFECTS

Sample	Excess Density (p.p.m.)	Sample	Excess Density (p.p.m.)
1. Oxygen-18 methanol	498	4. Oxygen-18 methanol	463
2. Oxygen-18 methanol	497	5. Oxygen-18 methanol	463
3. Oxygen-18 methanol	—	6. Oxygen-18 methanol	—

On raising the temperature of the nickel catalyst, prepared at 200 °C using a decomposition time of 21 hr, an initial rapid decrease in surface area occurred, but after 3–5 hr, the decrease in area with time became very much slower. After 20 hr at 275, 300, 320, and 340 °C the surface area was reduced to 0·46, 0·35, 0·20, and 0·04 respectively times the initial value at 200 °C. As surface area and catalytic activity are interrelated, these figures confirm the observation that the most active catalysts are obtained by decomposing the nickel compound at as low a temperature as possible.

It is found that a freshly prepared catalyst contains an appreciable amount of oxygen but most of this can be removed by reduction with hydrogen at 300–320 °C. It is possible to make a rough estimate of the amount of exchangeable oxygen associated with a catalyst from the memory effects shown by the data in Table 1. One millimole of carbon monoxide was reduced in each case and the weight of the nickel catalyst was 1·84 g. Calculation shows that, as an average of three results, $8{\cdot}0\times10^{-5}$ g atoms of oxygen were present on the catalyst. The surface area of this catalyst was approximately 2·6 by 10^4 cm^2. Hence, if the exchangeable oxygen is present as a chemisorbed layer one atom in thickness, the mean area occupied per oxygen atom or possibly ion is $5{\cdot}3\times10^{-16}$ cm^2. This figure is in apparent approximate agreement with the cross-sectional area for the oxygen ion O^{2-} calculated from the crystal radius $1{\cdot}40\times10^{-8}$ cm, as given by Pauling (1944). This apparent agreement is not stressed here but it probably supports the assumption made above that the

amount of oxygen remaining on the catalyst after treatment with hydrogen corresponds to a film about one atom (or ion) in thickness. Considering the stability of chemisorbed oxygen films on metals, it is probable that the experimental procedure used could not be expected to yield a catalyst with less oxygen than that which corresponds to one atomic layer and hence which would show less memory effect than that reported here.

It is of interest to note that Allen and Scaife (1954) report that an X-ray examination of the nickel obtained by the decomposition of the oxalate did not reveal the presence of nickel oxide. On the other hand, Boullé and David (1956) claim that X-ray diagrams of the solid obtained by heating nickel oxalate did show broad and indistinct lines of nickel and nickel oxide.

IV. Acknowledgments

The authors wish to thank Mr. B. Zerner for assistance with the experimental work. The surface area measurements were carried out by Mr. R. Clarke. Dr. J. A. Allen kindly supplied the pure nickel oxalate.

V. References

Allen, J. A., and Scaife, D. E. (1954).—*J. Phys. Chem.* **58**: 667.
Anbar, M., Dostrovsky, I., Klein, F., and Samuel, D. (1955).—*J. Chem. Soc.* **1955**: 155.
Bircumshaw, L. L., and Edwards, J. (1950).—*J. Chem. Soc.* **1950**: 1800.
Boullé, A., and David, R. (1956).—*C.R. Acad. Sci. Paris* **243**: 495.
Brandner, J. D., and Urey, H. C. (1945).—*J. Chem. Phys.* **13**: 351.
Brunauer, S., Emmett, P. H., and Teller, E. (1938).—*J. Amer. Chem. Soc.* **60**: 309.
Bunton, C. A., and Wood, J. L. (1955).—*J. Chem. Soc.* **1955**: 1522.
Herbert, J. B. M., and Lauder I. (1938).—*Trans. Faraday Soc.* **34**: 432.
Kudryavtsev, R. V., Ottesen, B. V., and Kursanov, D. N. (1956).—*Zh. Obshch. Chim.* **26**: 1035.
Lauder, I. (1959).—*Aust. J. Chem.* **12**: 32.
Lauder, I., and Wilson, I. (1959).—*Aust. J. Chem.* **12**: 29.
Lauder, I., and Zerner, B. (1959).—*Aust. J. Chem.* **12**: 621.
Meulen, H. ter (1922).—*Chem. Weekbl.* **19**: 191.
Pauling, L. (1944).—"The Nature of the Chemical Bond." p. 346. (Cornell Univ. Press.)
Roberts, R. M., and Madison, J. J. (1957).—*Analyt. Chem.* **29**: 1555.
Russell, W. W., and Fulton, J. W. (1933).—*Industr. Engng. Chem. Anal.* **5**: 384.
Urry, G., and Urry, W. H. (1956).—*Rev. Sci. Instrum.* **27**: 189.

THE CONVERSION OF OXYGEN IN COMPOUNDS TO CARBON MONOXIDE FOR MASS-SPECTROMETRIC ANALYSIS

By I. LAUDER* and B. ZERNER*

[*Manuscript received April* 28, 1959]

Summary

A method by which oxygen in many organic compounds and some inorganic compounds can be converted to carbon monoxide for mass-spectrometric analysis is described. The compound is decomposed in the vapour state in the presence of excess bromine by contact with a clean or a carbon-coated platinum wire at a bright red heat. The bromine acts as a scavanger and removes hydrogen and hydrocarbons. Carbon monoxide is obtained quantitatively and may be isolated in a pure state, except when the compound contains nitrogen, by freezing out the other decomposition products using liquid air. Memory and dilution effects are very small.

I. INTRODUCTION

Studies involving the use of oxygen-18 as a tracer have become very widespread since this isotope became available commercially, and numerous analytical techniques have been developed for the estimation of the oxygen-18 content of compounds. The first " general " method for isolating oxygen from organic compounds in a form suitable for mass-spectrometric analysis was introduced by Doering and Dorfman (1953). The compound is pyrolysed by contact with carbon at 1120 °C. The carbon monoxide formed is then oxidized by iodine pentoxide to carbon dioxide and this gas is led into the mass spectrometer. In this method, Bender and Kemp (1957) found it necessary to correct for a " blank " which is due to a reaction between the carbon and the quartz tube. Bender, Stone, and Dewey (1956) state the blanks are never over 10 per cent., and usually under 5 per cent. Bunton and co-workers (Bunton and Konasiewicz 1955 ; Bunton, Lewis, and Llewellyn 1956 ; Bunton and Spatcher 1956) use the first step of the above procedure, in a slightly different form, in their method of analysis. The organic compound is pyrolysed by passage through a carbon tube heated by radio-frequency current to 1200 °C. The gases formed are passed into the mass spectrometer and isotopic analysis is made on the carbon monoxide present. No details are given nor are the factors affecting the accuracy of the method discussed.

A procedure for the determination of the isotopic composition of oxygen in alcohols and related compounds has been described by Anbar *et al.* (1955) but methanol and various other compounds could not be analysed by the technique. Calibration experiments are required to allow for incomplete exchange and isotope effects.

* Department of Chemistry, University of Queensland, Brisbane.

The pyrolysis of methanol and ethanol by circulation in the vapour state over a white-hot platinum filament as described by Lauder and Wilson (1959) was tried by the present authors, as a means of producing carbon monoxide for mass-spectrometric analysis. The gas produced was cooled to −183 °C to freeze out condensable material and then led into the mass spectrometer. However, variable amounts of ethane were shown to be present in the products of pyrolysis and the mass 30 ion current was up to 25 per cent. greater than that expected for the $^{12}C^{18}O$ from an alcohol of normal isotopic composition. As carbon is deposited on the platinum filament during pyrolysis, it did not appear that the technique used by Bunton and co-workers (loc. cit.) would be successful in producing a gas free from ethane. The simple pyrolysis method was therefore judged to be not sufficiently reliable for accurate ^{18}O-analysis but, by a slight modification, the oxygen in many volatile organic compounds and some inorganic compounds can be readily isolated quantitatively as carbon monoxide in the pure state, except when nitrogen is present.

The method is described in detail below. In brief, the compound is decomposed in the vapour state in the presence of excess bromine by contact with a clean or carbon-coated platinum wire at a bright red heat. The bromine acts as a scavenger and removes hydrogen and hydrocarbons. The carbon monoxide is isolated by freezing out all other decomposition products and excess bromine.

II. Experimental

(*a*) *Materials*

The organic compounds, except those enriched in oxygen-18, were purified by standard laboratory procedures. The methanol and ethanol enriched in oxygen-18 were obtained by hydrolysis of dimethyl and diethyl sulphate respectively in oxygen-18 water. The procedure finally adopted for the purification of bromine is as follows: Bromine A.R. was shaken with sulphuric acid A.R. for 7 days and then distilled from sulphuric acid A.R. using a fractionating column 30 in. long, filled with glass helices. A middle fraction was selected and fractionated again (without sulphuric acid) in the same column, b.p. 58·7 °C at 761 mm Hg (lit. b.p. 58·78 °C ±0·03, 769 mm Hg; *International Critical Tables* (1928) **3**: 201). The bromine was then passed, in the vapour state, over anhydrous calcium sulphate.

As bromine is an unpleasant substance to handle, the amount required for each decomposition is stored in a small tube provided with a breakable tip. These tubes are filled by vacuum manipulation and sealed off immediately the bromine is purified. The glass tube from which the sample tubes are made should be cleaned thoroughly before the tubes are constructed as otherwise very fine leaks are occasionally found at the sealed-off tip.

(*b*) *Method*

A diagram of the experimental set-up is shown in Figure 1. Three different forms of "crackers", made from Pyrex glass, have been used. These are illustrated in Figure 2. For volatile compounds such as the lower aliphatic alcohols, acetone, ether, etc. type *A* cracker is used. Tungsten wires (0·040 in.) butt-welded to nickel are used as lead-in wires. The tungsten sealing glass at

the pinch (Osram-G.E.C. C9) should not cover the butt-weld. The platinum filament wire is spot-welded to the nickel or merely squeezed between the nickel. Sample tubes containing the compound and bromine respectively are attached to the B14 joints shown in Figure 1. These joints and stopcocks *A* and *B* are greased with fluorocarbon grease " Florube grease W ".* The system is evacuated well while the platinum filament in the cracker is maintained at a red heat and the glass walls of the cracker are heated (150–200 °C) with a flame. Stopcock *B* is protected by asbestos paper from the flame and cooled by a jet of air. The alcohol and the bromine in turn are transferred to the cracker by use of liquid air. Stopcock *B* is turned off. The liquid air is removed. When the bromine

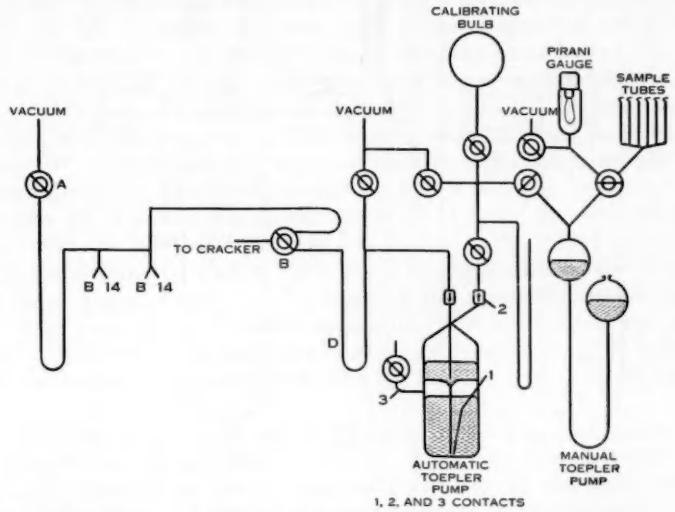


Fig. 1.—Apparatus used for manipulating the reagents and for transferring the carbon monoxide formed to sample tubes.

begins to liquefy, the walls of the cracker are warmed (*c.* 60 °C) with a flame to convert most of the bromine to the gaseous state. The filament current is then turned on. With a platinum wire 0·008 in. diameter and a current of 4·0–4·5 A, the temperature reached is about 1100 °C. Pyrolysis appears to be complete within a few minutes judging from yields, but the filament is usually heated for 7–10 min. The bromine acts as a scavenger and " mops up " hydrogen and hydrocarbons. The cracker is cooled in liquid air to freeze out the condensable material—hydrogen bromide, bromine, and carbon–bromide compounds. The U-bend *D* is also cooled in liquid air. The carbon monoxide is pumped by means of the automatic Toepler (Urry and Urry 1956; Roberts and Madison 1957) into the manual Toepler. The latter is then used to compress the gas to 60–70 cm Hg pressure into sample tubes, which are then sealed off.

* I.C.I. product.

Very little attack by bromine or the decomposition products on the nickel portion of the tungsten–nickel lead-in wires occurs during use of the cracker. However, attack does occur if moisture is allowed to mix with the decomposition products. For this reason, after the cracker has been used and the carbon monoxide has been pumped out, the volatile material frozen in the cracker is transferred immediately to one of the tubes attached to the B14 joints and is

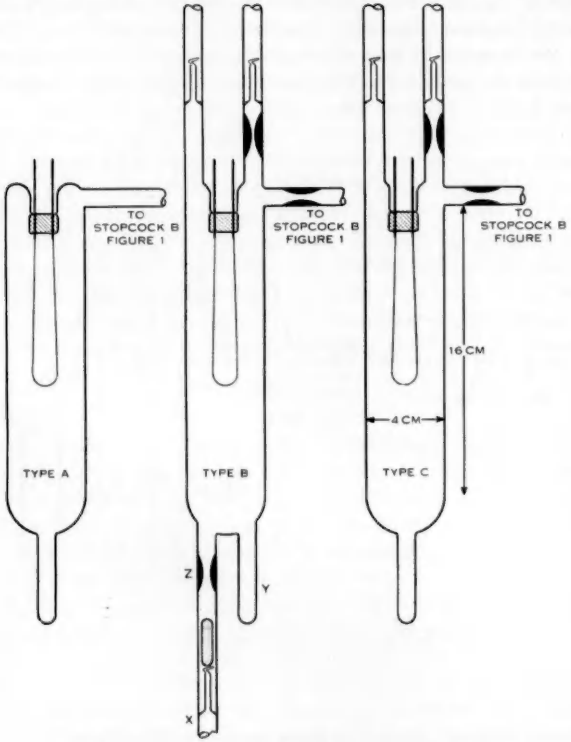


Fig. 2.—Diagrams illustrating types of crackers used.

removed from the system. A small bulb of known volume and a manometer are attached to the system on the compression side of the automatic Toepler for the purpose of determining the yield of gas when any new compound is treated.

The cracker is cleaned with chromic acid–sulphuric acid mixture at 130 °C after 3–4 crackings, and it may be used for a total of 20–25 times or more before replacement of the filament is necessary.

Compounds which are not very volatile or which yield water or carbon dioxide as a decomposition product are treated in the type *B* cracker. The conversion of the oxygen in any water or carbon dioxide formed is achieved by reaction with carbon at 1100 °C deposited on the platinum wire. Some organic

compounds such as benzhydrol will deposit sufficient carbon themselves on the wire during pyrolysis to allow the conversion to take place. However, when this is not the case, adequate carbon can be deposited by adding some naphthalene to the reaction mixture. The compositions of the reaction mixtures used in the present work are shown in Table 1. Solid materials or liquids of low volatility are placed in a small glass tube, and slipped into the cracker through the tube *Y* which is then sealed off. The system is evacuated and bromine is introduced as before. The cracker is then sealed off and placed in a tube furnace. The temperature is raised to 350 °C and, after 10 min, the filament current is adjusted to keep the wire at a bright red heat—9 A for platinum wire 0·013 in. in diameter. Pyrolysis and formation of the carbon layer on the filament occurs. After 30 min the cracker is joined once again to the vacuum line. Condensable material is frozen out, and the carbon monoxide isolated as before.

If it is desired to eliminate as far as possible isotopic contamination from all sources, two decompositions may be carried out in succession. Use is made of the carbon on the platinum wire and the bromine remaining from the first decomposition. The second sample of oxygen-containing compound is introduced by vacuum sublimation through the breakable seal at *X* while the bromine is frozen in the tube *Y*. The tube *X*-*Z* is then sealed off at *Z* and the decomposition is brought about as previously described.

A heavier platinum wire, 0·013 in., is used in type *B* and also in type *C* cracker than is used in type *A*. The reason for this is that the smaller diameter platinum wire covered with carbon occasionally fails after one or two decompositions. The commercial grade of wire with a minimum purity of 99·9 per cent. shows less tendency to fracture than the thermo-pure wire of 99·99 per cent. purity.

In crackers of types *B* and *C*, tungsten–nickel–platinum lead-in wires (0·040 in. dia.) are used and only the platinum projects from the press seal. This construction prevents the nickel from being attacked by the bromine at the elevated temperatures at which the vessels are used but it introduces another problem. The tungsten sealing glass is not in intimate contact with the nickel and the platinum, and careful outgassing is required to remove any water etc. trapped in this space.

For the conversion of water or carbon dioxide to carbon monoxide, a cracker of type *C* is used. The method of use is similar to that already described for type *B*. Provision is made for carrying out two decompositions in succession. No bromine is required for the conversion of carbon dioxide to carbon monoxide but bromine is required initially in order to deposit carbon from the naphthalene on the wire. If the naphthalene is pyrolysed in the absence of the bromine the rate of decomposition is very much slower than in the presence of bromine and very little carbon deposits on the wire.

III. Results

Results are shown in Table 1. The values in the final column were obtained with a Nier-type isotope-ratio mass spectrometer. The figures have not been corrected for instrumental factors (which are small), for differences in rates of

diffusion out of the ion source, nor for the simultaneous collection of mass 29 ($^{13}C^{16}O$, $^{12}C^{17}O$) with mass 28 ($^{12}C^{16}O$). Compounds not prefixed by ^{18}O-

TABLE 1

YIELDS AND MASS-SPECTROMETRIC ANALYSES OF CARBON MONOXIDE FROM ORGANIC AND SOME INORGANIC COMPOUNDS

Compound	Amount of Compound (mmole)	Amount of Bromine (mmole)	Amount of Naphthalene (mmole)	Type of Cracker	Yield of Carbon Monoxide (%)	30/28 Ratio $\times 10^5$
Methanol ..	1·0	—	—	*A*	100	216
Methanol ..	1·0	10	—	*A*	100	209
^{18}O-methanol ..	1·0	—	—	*A*	100	642
^{18}O-methanol ..	1·0	10	—	*A*	100	632
Ethanol	1·0	—	—	*A*	60	260
Ethanol	1·0	10	—	*A*	100	208
^{18}O-ethanol ..	1·0	—	—	*A*	60	825
^{18}O-ethanol ..	1·0	10	—	*A*	100	703
2-Propanol ..	1·0	—	—	*A*	50	—
2-Propanol ..	1·0	10	—	*A*	96	212
tert.-Butanol ..	1·0	—	—	*A*	0	—
tert.-Butanol ..	1·0	10	—	*A*	75	—
tert.-Butanol ..	1·0	10	0·9	*B*	100	212
Acetone	1·0	10	—	*A*	100	214
Diethyl ether ..	1·0	10	—	*A*	100	215
Benzhydrol ..	1·0	10	—	*B*	96	213
Phenol	1·0	10	0·4	*B*	73	213
Benzoic acid ..	0·4	5	—	*B*	12	207
	0·4	3	0·2	*B*	33	207
	0·4	4	0·5	*B*	100	209
Diethyl sulphate	0·21	5	0·7	*B*	96	217
Benzamide ..	0·34	5	0·25	*B*	58	170
	0·25	5	0·45	*B*	52	182
^{18}O-water ..	0·5	5	0·5	*C*	100	724
^{18}O-carbon dioxide	0·1	5	0·4	*C*	100	768

were of " normal " isotopic composition. The slight variations in the measured 30/28 ratios for normal compounds, except for the first results for methanol and ethanol respectively, are possibly related to the sources of oxygen—air or water—

used in the manufacture of the compounds. The oxygen-18 content of natural water is a few per cent. less than that of atmospheric oxygen (Dole 1952).

The efficiency of bromine as a scavenger for hydrocarbons can be judged from the results with methanol and ethanol. When the decomposition is carried out in the presence of bromine, no extraneous background is observed and in the case of ethanol the yield of carbon monoxide becomes quantitative.

The necessity for depositing carbon on the platinum filament in certain cases is illustrated by the results with *tert.*-butanol and also with benzoic acid. If *tert.*-butanol is circulated in the vapour state over a clean platinum filament at a low red heat an almost quantitative yield of water may be obtained (unpublished data from work with Mr. V. R. Stimson). When type *B* cracker is used and decomposition is brought about in the presence of bromine but with no carbon specially deposited on the filament, the yield of carbon monoxide is only 75 per cent. theoretical. In the presence of carbon the yield is quantitative. With benzoic acid a similar rise in yield is observed as more carbon is deposited on the wire.

When the naphthalene is added to the reaction mixture it should be noted that not all the carbon in the added naphthalene is deposited on the wire. The upper portion of the surface of the reaction vessel becomes covered with carbon during the decomposition. It was found that 1 mmole of naphthalene gave about 2·5 mg atoms of carbon on a filament 13 to 16 cm long. Some compounds deposit carbon on the filament more easily than others. Benzhydrol appeared to be more effective in this respect than naphthalene but the use of this compound as a source of carbon is ruled out because oxygen is present in the compound. α-Pinene seemed to give about the same amount of carbon as naphthalene.

Mass-spectrometric analysis of the gas isolated from the decomposition of normal benzamide showed that only mass numbers 28, 29, and 30 were present. Some deviation from the expected ratio for carbon monoxide to nitrogen, namely 2:1, could be accounted for by the different ionization efficiencies of the two gases (Barnard 1953), but even allowing for this, the mixture is richer in mass 30 than expected. Possibly a trace of nitric oxide, mass 30, is formed. In addition the yield is poor. Further investigation of the problems arising with the decomposition of benzamide is required.

The results for diethyl sulphate show that the oxygen in sulphur dioxide and also in the trioxide could be obtained in the form of carbon monoxide.

The conversion of oxygen in water to carbon monoxide is quantitative because, under the experimental conditions, virtually all hydrogen is removed by the bromine. Again, conversion of carbon dioxide to carbon monoxide is virtually quantitative. The equilibrium partial pressure of carbon dioxide under the experimental conditions is very low (Rhead and Wheeler 1911).

Accuracy and Dilution Effects.—When oxygen-18 methanol and ethanol are converted in type *A* cracker there appears to be little if any isotopic dilution. Duplicate samples from different lots of oxygen-18 methanol and ethanol were decomposed and the 30/28 ratios were measured in the mass spectrometer under the same operating conditions. Results are shown in Table 2. In the first column, letters *A* and *C* indicate the type of cracker used. The experiment with

ethanol, sample No. 4C, was carried out to determine whether the result obtained under conditions designed to minimize isotopic dilution effects was significantly different from that obtained by the simple technique with type *A* cracker. A blank experiment was first carried out. 0·75 mmole of oxygen-18 ethanol and 10 mmole of bromine were sealed in a type *C* cracker. The vessel was then placed in a tube furnace and heated to 500 °C. The platinum wire was maintained at a bright red heat. After 1 hr under these conditions the vessel was sealed to a vacuum line and the hydrogen bromide and carbon monoxide were removed, leaving only bromine in the vessel. Then 0·8 mmole of oxygen-18 ethanol was added and the vessel was sealed again. Six mmole of bromine remained from the blank experiment. Decomposition was brought about by the platinum

TABLE 2

RESULTS SHOWING THE ABSENCE OF ISOTOPIC DILUTION EFFECTS

Compound and Sample Number	Amount of Compound (mmole)	Amount of Bromine (mmole)	Time of Cracking (min)	30/28 Ratio $\times 10^5$
^{18}O-methanol				
1A.. ..	1	10	7	609·5
2A.. ..	1	10	7	607·3
3A.. ..	1	10	7	634·0
4A.. ..	1	10	7	632·0
5A.. ..	1	10	7	730·7
6A.. ..	1	10	7	732·5
^{18}O-ethanol				
1A.. ..	1	10	12	587·5
2A.. ..	1	10	12	589·0
3A.. ..	0·75	10	12	723·5
4C.. ..	0·8	6	20	724·5

wire at bright red heat while the vessel was maintained at 200 °C in the tube furnace. After 20 min, the carbon monoxide was isolated as previously described. The agreement between the results for samples Nos. 3A and 4C shows that if any isotopic dilution occurs with the procedure outlined for use of type *A* cracker, it is very small indeed. In every case, the agreement between results for duplicate samples treated in type *A* cracker is good.

During the conversion of water enriched in oxygen-18 there is a loss amounting to about 3 per cent. for a 0·5–0·8 mmole sample containing 0·7 atom per cent. oxygen-18 when the temperature of the cracker is maintained at 500 °C during decomposition. Small amounts of normal oxygen can gain access to the system from several sources, but for accurate work these may be eliminated or reduced to very small proportions by the following steps: (i) Carry out one decomposition at 500 °C as described above, depositing sufficient carbon on the wire for two conversions. (ii) Remove all volatile materials and introduce oxygen-18 water to give about 1 atm pressure at 250 °C. Heat at this temperature for 1 day. (iii) Evacuate the vessel, return the bromine remaining from

the first decomposition, and add the sample of water to be converted. (iv) Carry out the conversion at 150 °C. Care must be exercised to avoid isotopic contamination of the water sample and the bromine during the vacuum manipulations. All glass surfaces must be equilibrated with oxygen-18 water of the same composition as is being decomposed.*

The above procedure and a similar one for the conversion of carbon dioxide to carbon monoxide have been used to study the fractionation factor for the exchange of oxygen between carbon dioxide and liquid water at 25 °C. All isotope-ratio measurements are made on carbon monoxide. This work will be reported elsewhere.

IV. Acknowledgment

One of us (B.Z.) acknowledges the award of a research scholarship by the Australian Atomic Energy Commission.

V. References

Anbar, M., Dostrovsky, I., Klein, F., and Samuel, D. (1955).—*J. Chem. Soc.* **1955**: 155.
Barnard, G. P. (1953).—"Modern Mass Spectrometry." pp. 65, 87. (Institute of Physics: London.)
Bender, M. L., and Kemp, K. C. (1957).—*J. Amer. Chem. Soc.* **79**: 117.
Bender, M. L., Stone, R. R., and Dewey, R. S. (1956).—*J. Amer. Chem. Soc.* **78**: 319.
Boggs, J. E., and Mosher, H. P. (1956).—*J. Amer. Chem. Soc.* **78**: 3901.
Bunton, C. A., and Konasiewicz, A. (1955).—*J. Chem. Soc.* **1955**: 1354.
Bunton, C. A., Lewis, T. A., and Llewellyn, D. R. (1956).—*J. Chem. Soc.* **1956**: 1226.
Bunton, C. A., and Spatcher, D. N. (1956).—*J. Chem. Soc.* **1956**: 1079.
Doering, W. von E., and Dorfman, E. (1953).—*J. Amer. Chem. Soc.* **75**: 5595.
Dole, M. (1952).—*Chem. Rev. Lond.* **51**: 263.
Lauder, I., and Wilson, I. R. (1959).—*Aust. J. Chem.* **12**: 613.
Rhead, T. F. E., and Wheeler, R. V. (1911).—*Chem. Soc. Trans.* **99**: 1140.
Roberts, R. M., and Madison, J. J. (1957).—*Analyt. Chem.* **29**: 1555.
Urry, G., and Urry, W. H. (1956).—*Rev. Sci. Instrum.* **27**: 819.

* Further work with Mr. B. Fleischfresser has shown that the hydrogen bromide formed during the process brings about an isotopic dilution of the water sample undergoing conversion. This dilution, due presumably to oxygen derived from the glass surface of the reaction vessel, amounts to 1–2 per cent. for an 0·4 mmole water sample, depending on experimental conditions (cf. Boggs and Mosher 1956).

THE MASS-SPECTROMETRIC ANALYSIS OF OXYGEN IN CARBON MONOXIDE AND CARBON DIOXIDE

By I. LAUDER*

[*Manuscript received April* 28, 1959]

Summary

Results of measurements of oxygen-18 abundances in carbon monoxide and carbon dioxide using a Nier-type isotope-ratio mass spectrometer are given. Carbon monoxide is a far superior gas to carbon dioxide as a carrier for the oxygen isotopes because of the more rapid pump-out and almost complete absence of memory in the mass spectrometer. The slight variation of the measured ratio with output voltage is attributed mainly to the non-ohmic response of the grid resistors to the electrometer valves.

I. INTRODUCTION

If suitable methods are available for converting the oxygen in a compound either to carbon monoxide or to carbon dioxide, the question arises as to which gas is the better for mass-spectrometric analysis. This can be decided by considering the pump-out times and memory effects for the two gases.

Kirshenbaum (1951) has discussed certain operational effects and memory effects for hydrogen isotopes in mass spectrometers but for other isotopes no information on this subject is to be found in the literature, although it is essential for accurate abundance work. For this reason, data obtained in this laboratory on the measurement of oxygen-18 concentrations in carbon dioxide and also in carbon monoxide using a Nier-type isotope-ratio mass spectrometer are presented here.

II. DESCRIPTION OF INSTRUMENT

The mass spectrometer was constructed in this Department and is of the same basic design as that described by Nier (1947) except for some minor modifications. The ion source is of the type described by Nier (1950). One hundred per cent. inverse feed back amplifiers described by Nier (1947) are used, with grid resistors to the electrometer valves each $4 \times 10^{10}\ \Omega$. A potential of 70 V accelerates the electrons producing ionization and a trap current of 75 μA is maintained. The potential used to accelerate the positive ions into the magnetic field is generally in slight excess of 2000 V. Under operating conditions, the pressure measured about the cold trap is in the region 10^{-7} mm Hg.

A double gas-handling system is employed. This facilitates comparisons between gases of known and unknown composition and allows small differences in composition to be determined more readily. The taps are greased with

* Department of Chemistry, University of Queensland, Brisbane.

"Silicone" grease. When cleaning is necessary, carbon tetrachloride is used as this compound does not give rise to mass numbers 30 or 46. Originally a variable copper spiral leak was installed (Nier, Ney, and Inghram 1947), but this form of leak appeared to have an appreciable memory effect for carbon dioxide. It was replaced by a glass-capillary leak constructed from a piece of constant bore tube 0·1 mm diameter by 14·5 cm long. The capillary was further constricted at the ion-source end (Nier 1948) so that an upstream pressure for carbon monoxide of 3–4 mm Hg is required to give an output of 20 V due to mass 28. After outgassing the metal analyser tube at 300 °C the background due to mass 28 falls to about 8 mV; that due to mass 44 to less than 1 mV, and that due to water to less than 2 mV, depending on the use to which the apparatus has been placed prior to outgassing.

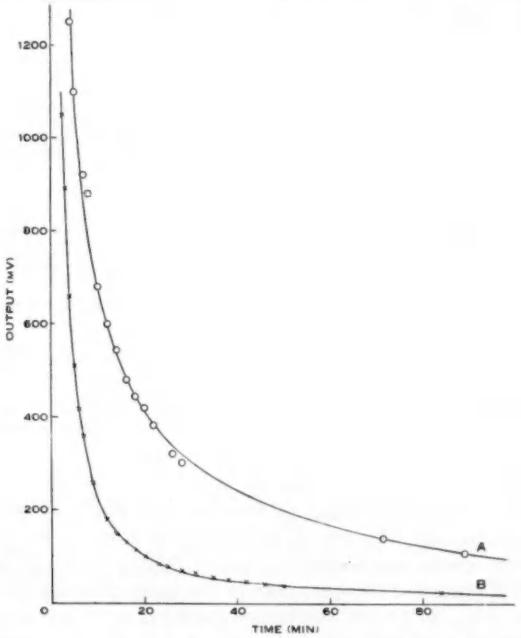


Fig. 1.—*A*, rate of pump-out for carbon dioxide; *B*, rate of pump-out for carbon monoxide.

III. Results

(*a*) *Pump-Out Time*

The curves in Figure 1 show plots of amplifier outputs against time for carbon monoxide and also for carbon dioxide during pump-out after the gases had been in the mass spectrometer for 3 and 3½ hr at pressures giving 30 V outputs for masses 28 and 44 respectively. Carbon monoxide is removed about four times as fast as carbon dioxide.

(*b*) *Memory Effect*

The memory effect depends on the differences in isotopic abundance for successive samples. For carbon monoxide the results are shown in Figure 2. The experimental conditions for curve *A* were as follows: Normal carbon monoxide, at a pressure sufficient to give an output of 20–30 V for mass 28 flowed through the spectrometer for 3 hr. This was followed by a pump-out for 109 min during which time the mass 28 output fell to 21 mV. Oxygen-18 carbon monoxide was then admitted. Within 5 to 7 min of letting the gas into the spectrometer, the measured ratio was less than 1 per cent. different from the equilibrium value and in 15 min the equilibrium value for the ratio 30/28, namely, 0·00736, was reached.

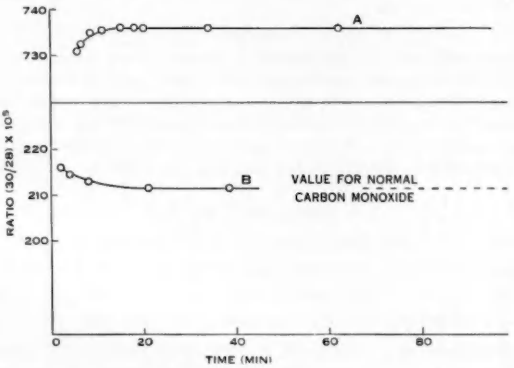


Fig. 2.—*A*, approach to equilibrium for oxygen-18-enriched carbon monoxide following normal carbon monoxide in the spectrometer; *B*, approach to equilibrium for normal carbon monoxide following oxygen-18 carbon monoxide used to determine curve *A*.

When carbon monoxide of normal isotopic composition follows carbon monoxide with a ratio 30/28 equal to 0·00736, the return to the normal value takes about 15 min. Results are shown in Figure 2, curve *B*. The conditions under which this curve was obtained were as follows: The oxygen-18 carbon monoxide flowed through the spectrometer for approximately 95 min and then it was pumped out. In 24 min the background fell to 72 mV of mass 28. The normal gas was then introduced.

The approach to equilibrium when carbon dioxide is introduced is very much slower. Results are shown in Figure 3. The experimental conditions under which curve *A* was obtained were as follows: Carbon dioxide of normal isotopic composition flowed through the spectrometer for 3½ hr. This gas was then pumped out and after 90 min the background due to mass 44 was 112 mV. The oxygen-18-enriched carbon dioxide was then introduced. These experimental conditions are almost identical with those under which curve *A* of Figure 2 for carbon monoxide was obtained and the atom per cent. oxygen-18 was practically the same in the two cases.

After 60 min, the measured ratio had approached within about 1 per cent. of the equilibrium value and it took about another 130 min for the 46/44 ratio to reach a value which remained more or less constant with time. At the end of this period the oxygen-18 carbon dioxide was pumped away. After 80 min the mass 44 background had fallen to 130 mV. Carbon dioxide equilibrated with tap water was then introduced. The change in the measured ratio is shown in Figure 3, curve *B*. After 1 hr the measured ratio is 3 per cent. greater than the normal value.

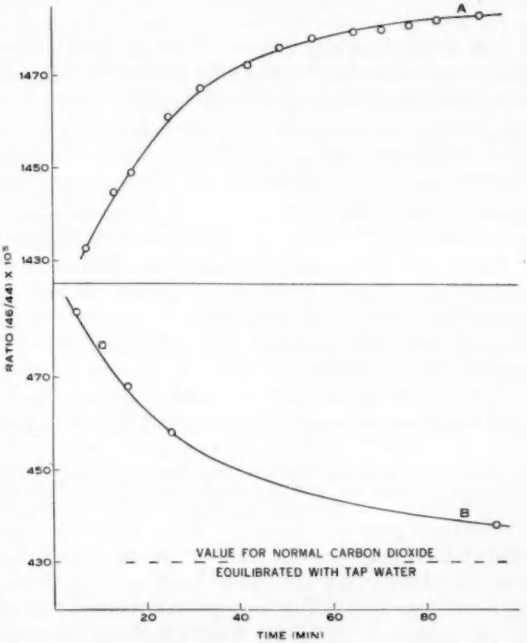


Fig. 3.—*A*, approach to equilibrium for oxygen-18-enriched carbon dioxide following normal carbon dioxide in the spectrometer; *B*, approach to equilibrium for normal carbon dioxide used to determine curve *A*.

(c) *Variation of Measured Ratio with Output Voltage*

An effect which has not so far been mentioned is the variation in measured ratio with output voltage. For carbon dioxide containing 0·740 atom per cent. oxygen-18, a plot of measured ratio 46/44 against output voltage for mass 44 is shown in Figure 4.

The deviation of the measured ratio from the straight-line value at the lower end of the plot is attributed to fractionation effects in the capillary leak through which the gas enters the mass spectrometer and to memory effects from the normal carbon dioxide previously in the spectrometer. The linear portion

of the graph can be represented by the equation

$$R_m = R_0 + aV_1, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where R_m is the measured voltage ratio V_2/V_1; V_2 is the output voltage from collector 2 and V_1 is the output voltage from collector 1; a is the slope of the line in volts^{-1}; R_0 is the ratio at zero output voltage. It is found that, within experimental error, a is a linear function of R_0 and thus equation (1) can be put into the form

$$R_m = R_0(1 + bV_1). \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

This equation was found to apply for all ratios measured using normal and oxygen-18-enriched carbon monoxide and also carbon dioxide. The constant b was found to have an average value $6 \cdot 8 \pm 0 \cdot 7 \times 10^{-4}$ V^{-1}.

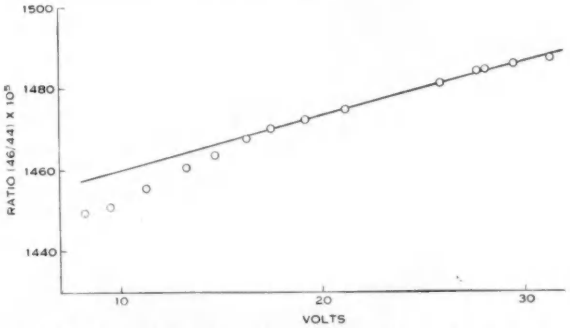


Fig. 4.—The variation of measured ratio $(46/44) \times 10^5$ with output voltage due to mass 44 for carbon dioxide containing 0·736 atom per cent. oxygen-18.

Brown (1956) also Craig (1957), both of whom were investigating fine variations of isotope abundances in carbon dioxide, using Nier-type spectrometers with dual collector systems, have reported increases in measured ratio with increasing gas pressure. Craig has attributed the effect to "a squared dependence of the mass-44 tail contribution to the less abundant ion beams". If the effect were due to a contribution by the tail of the intense beam to the rarer beam then the effect would become relatively less important, the greater R_0. This conclusion is contrary to equation (2) above. Again a tail contribution from the intense beam to the rarer beam would be more important the smaller the mass dispersion. However, for the same $^{13}C/^{12}C$ abundance, it was found that the variation in measured ratio is the same whether measurements are made on the 29/28 ratio of carbon monoxide or the 45/44 ratio of carbon dioxide. On these grounds Craig's view appears to be incorrect.

The suggestion is made here that the observed effect is due to non-ohmic responses of the high resistance grid leaks attached to the electrometer valves and possibly to phenomena associated with secondary electron emission in the collector system. If the resistances of the grid leaks vary with applied voltages,

and no other effects enter, it is easy to show that

$$R_m = R_0[1 + f(V_2) - f(V_1)],$$

where $f(V_2)$, $f(V_1)$ are functions which take into account the variations of resistance of the grid leaks across which the voltages V_2, V_1 respectively develop. According to the manufacturer's data, for the grid leaks presently in use, the resistances are linear functions of the applied voltages, the coefficient quoted being -3×10^{-4} V^{-1}. However, this figure represents a "typical value and is not intended to be interpreted as either a maximum value or a minimum value" (personal communication, Victoreen Inst. Co., Ohio). For isotope ratio measurements on carbon dioxide and carbon monoxide reported in this paper $V_1 \gg V_2$. Thus the above equation reduces to the form which accounts for the experimental results but the observed voltage coefficient is slightly more than twice the quoted value. As the work to which the mass spectrometer is being placed does not involve absolute abundance measurements, the expenditure of time on the measurement of voltage coefficient of resistance did not appear to be warranted.

The dual collector system used on the mass spectrometer is essentially of the same design as that given by Nier (1947). The collectors consist of plates above which are situated secondary electron suppressor plates maintained at -45 V with respect to ground. It is found that the variation of R_m with V_1 is not appreciably changed if the suppressor voltage is changed to -90 V. However, this observation does not eliminate the possibility that secondary electron effects are present in the collector system. If such effects are present, they could be considerably reduced by the use of Faraday cages for collecting the beams as suggested by Barnard (1953) and also by Ewald and Hintenberger (1953).

For isotope-tracer work where it is only necessary to determine the change in some abundance ratio the variation of R_m with V_1 is not of importance provided all measurements are made under the same operating conditions.

IV. Acknowledgments

It is a pleasure to acknowledge the assistance and advice of many colleagues during the construction of the mass spectrometer, especially Professor A. O. Nier, Department of Physics, University of Minnesota, Professor M. Shaw, Department of Mechanical Engineering, University of Queensland, and Mr. R. Hinkley and Dr. T. Parnell, Department of Electrical Engineering, University of Queensland. Mr. N. Unwin of this Department carried out most of the mechanical work associated with the construction, and Mr. D. Page-Hanify, Mr. W. Donaldson, and Mr. W. Moore constructed the electronic units under the supervision of Mr. W. Robson. The author wishes to acknowledge financial assistance from the Australian Atomic Energy Commission.

V. References

Barnard, G. P. (1953).—"Modern Mass Spectrometry." p. 123. (Institute of Physics: London.)

Brown, J. B. (1956).—"A Study of the Oxygen Isotopes." Doctoral Dissertation Series, Publ. No. 18969. (University Microfilm: Ann Arbor, Michigan.)

CRAIG, H. (1957).—*Geochim. et Cosmochim. Acta* **12**: 133.

EWALD, H., and HINTERBERGER, H. (1953).—"Methoden und Anwendungen der Massenspektroskopie." p. 191. (Verlag Chemie: Weinheim.)

KIRSHENBAUM, I. (1951).—"Physical Properties and Analysis of Heavy Water." National Nuclear Energy Series III–4A. (McGraw-Hill Book Co.: New York.)

NIER, A. O. (1947).—*Rev. Sci. Instrum.* **18**: 398.

NIER, A. O. (1948).—*Analyt. Chem.* **20**: 188.

NIER, A. O. (1950).—*Phys. Rev.* **77**: 789.

NIER, A. O., NEY, E. P., and INGHRAM, M. (1947).—*Rev. Sci. Instrum.* **18**: 191.

SOME STUDIES IN INORGANIC COMPLEXES

V. MERCURY(II)

By G. J. SUTTON*

[*Manuscript received May 7, 1959*]

Summary

A study has been made of mercury(II) complexes of ethylenediamine, 1,10-phenanthroline, and 2,2′-dipyridyl. By means of conductance and molecular weight measurements it has been shown that complexes of the type $HgX_2.B$ and HgY.B, in which B is the base, X is halogen or nitrite, and Y is oxalate or sulphate, are 4-covalent non-electrolytes. The absorption spectra of the halogen complexes in the near ultraviolet range are characteristic of the chelate with a shift of absorption to longer wavelengths. Bisphenanthroline and bisdipyridyl complexes of mercury(II) were also investigated.

I. INTRODUCTION

Previous studies of ethylenediamine complexes of mercury(II) involved the preparation of the complex $HgI_2 \cdot en$ by Straumaris and Circulis (1936) and $HgCl_2 \cdot en$ and $(HgCl_2)_2 \cdot en$ by O'Brien (1948). The existence of the species Hg_x^{2+}, in which x may be 2, 3, or 4, has been reported by Nyman, Roe, and Mason (1955) and the salt $Hgen_2(ClO_4)_2$ was described by Pfeiffer, Schmitz, and Bohm (1952). Watters and Mason (1956) have shown the existence of the ions $Hg \cdot enOH^+$ and $HgH \cdot en_2^{3+}$ in aqueous solution. In this work the monoethylenediamine derivatives have been studied. The complex $HgI_2 \cdot dipy$ was used by Morgan and Burstall (1930) for the estimation of 2,2′-dipyridyl, and Pfeiffer and Christeleit (1938) and Pfeiffer and Werdelmann (1950) have reported the existence of the 1,10-phenanthroline complex $Hgphen_3(ClO_4)_2$. Although the mercuric salts of strong acids are strong electrolytes and those of moderately strong acids have about the same conductivity as the acids themselves, e.m.f. measurements by Infeldt and Sillén (1946) indicate that nitro (or nitrito) and sulphato bonding may occur with the mercury(II) atom in solution. The low ionic dissociation of mercuric nitrite was also observed by Ley and Kissel (1899), and nitro complexes of mercury have been described by Rosenheim and Oppenheim (1901). Although mercury is more readily bonded by nitrogen, sulphur, or the less negative iodine atoms, it was decided to attempt the preparation of monoethylenediamine and monophenanthroline mercury(II) complexes in the presence of nitrite, sulphate, and oxalate ions. Since both Roloff (1894) and Schaefer (1905) have shown that the bisoxalatomercurate(II) ion may exist, it was thought that coordination by basic chelating agents, occupying two positions of the tetrahedral lattice, might feasibly leave sufficient residual charge on the mercury atom to effect coordination

* School of Chemistry, University of New South Wales, Granville College, N.S.W.

by the negative oxygen atoms forming nitro, sulphato, and oxalato complexes respectively. Since mercury favours coordination by nitrogen rather than by oxygen, it is likely that the compounds would be nitro derivatives rather than nitroso complexes. It was found that these complexes may be precipitated by direct combination in dilute aqueous solution and for some of the more soluble

TABLE 1

MOLECULAR CONDUCTIVITIES OF MERCURY(II) COMPLEXES IN NITROBENZENE AT 25 °C

Substance	Conductivity (Ω^{-1})	M Concentration ($\times 10^{-3}$)	Substance	Conductivity (Ω^{-1})	M Concentration $\times 10^{-3}$
$HgCl_2$·en	0·23	6·0	$Hg(NO_3)_2$·en ..	1·25	3·0
$HgBr_2$·en	0·35	2·9	$HgSO_4$·en	1·40	2·5
HgI_2·en	0·39	7·0	HgC_2O_4·en	1·0	3·4
$HgCl_2$·phen	0·53	2·4	$Hg(NO_3)_2$·phen ..	1·84	1·5
$HgBr_2$·phen	0·37	3·0	$HgSO_4$·phen ..	1·80	1·7
HgI_2·phen	0·40	5·8	HgC_2O_4·phen ..	0·55	3·2
$HgCl_2$·dipy	0·43	6·5	Hg·$phen_2(NO_3)_2$ ..	49·1	1·2
$HgBr_2$·dipy ..	0·49	6·3	Hg·$dipy_2(ClO_4)_2$ ..	52·3	0·6
HgI_2·dipy	0·49	7·2			

ethylenediamine complexes by concentrating the solution. The halogen complexes of ethylenediamine, 1,10-phenanthroline, and 2,2′-dipyridyl were obtained similarly, having the general formula HgX_2.B, in which X may be chlorine or bromine and B the respective chelating base. All of these complexes are white excepting the pale yellow iodo derivatives, which may be obtained by reacting

TABLE 2

MOLECULAR CONDUCTIVITIES OF MERCURY(II) COMPLEXES AT 25 °C

Substance	Conductivity (Ω^{-1})	M Concentration $\times 10^{-3}$ in Methanol	Conductivity (Ω^{-1})	M Concentration $\times 10^{-3}$ in Nitromethane
$Hg(NO_3)_2$·en ..	5·8	2·1	5·1	2·0
$HgSO_4$·en ..	6·8	2·2	5·5	1·6
HgC_2O_4·en ..	4·3	2·2	3·7	3·6
$Hg(NO_3)_2$·phen ..	5·8	1·4	4·8	1·8
$HgSO_4$·phen ..	4·6	1·4	4·0	1·3
HgC_2O_4·phen ..	3·2	1·3	2·5	2·4

with the soluble tetraiodomercurate ion. The structures of all of these complexes were verified as 4-covalent non-electrolytes by conductivity measurements in nitrobenzene and the results are given in Table 1. In the case of the oxygen bonded complexes, conductivity measurements were also carried out in nitromethane and in methanol and the results are summarized in Table 2. It is noteworthy that although there was some conductance in the latter solvents the

values were less than 10 Ω^{-1}, whereas uni-univalent electrolytes have conductances in the vicinity of 60 and 105 Ω^{-1} in these solvents respectively. From these results it may be concluded that they are 4-covalent non-electrolytes, the slight conductances being due to some impurity or dissociation. For reasons of low solubility, molecular weight measurements were indeterminable in the aforementioned solvents. However, measurements were possible by the ebullioscopic method in the case of the oxalato and iodo complexes using ethylenedibromide as solvent, and the results are given in Table 3. These results, whilst indicating that some dissociation has taken place, show that the complexes are monomeric. The low solubilities of these complexes in solvents which may coordinate by electron pairs on oxygen atoms indicate that under these conditions the maximum covalency of mercury(II) is 4. The formation of a trisethylenediamine mercury complex by Weitz, Blasberg, and Wernicke (1930) and the trisphenanthroline mercury complex of Pfeiffer and co-workers involve the use of the less negative nitrogen atoms only in the coordination sphere. Although

TABLE 3

MOLECULAR WEIGHTS OF MERCURY(II) COMPLEXES IN ETHYLENEDIBROMIDE

Substance	Found	Calc.	Conc. (%)	Substance	Found	Calc.	Conc. (%)
$HgI_2 \cdot en$ ..	506	515	1·2	$HgC_2O_4 \cdot en$ ..	310	349	1·3
$HgI_2 \cdot phen$ ..	585	635	1·4	$HgC_2O_4 \cdot phen$ ..	421	469	1·4
$HgI_2 \cdot dipy$ ..	563	611	1·4				

these may be 6-covalent octahedral complexes, it would appear that this is only possible when the majority of the donor atoms have relatively low negativity. This would explain why the complexes described in this work are appreciably soluble in pyridine and in α-picoline in which 6-covalency may result with the electrons of the nitrogen atom of the solvent as the donor, although replacement by solvent molecules is also possible. The increased solubility of tetrammine mercuric perchlorate in aqueous ammonia may be explained similarly.

The addition of excess phenanthroline or dipyridyl to mercuric nitrate or perchlorate in concentrated solution resulted in the precipitation of the white bischelate complexes $Hg \cdot phen_2(NO_3)_2$ and $Hg \cdot dipy_2(ClO_4)_2$ respectively. These were shown to be bi-univalent electrolytes having a conductivity of about 50 Ω^{-1} in nitrobenzene (see Table 1).

The absorption spectra of the halide complexes were studied in the near ultraviolet region and it was found that the phenanthroline and dipyridyl complexes gave spectra which are characteristic of the ligands but with a displacement to longer wavelengths. Ethylenediamine having no double bonds gives no absorption in the region down to 2180 Å, although Scheibe (1929) has given figures for halide ion absorption in the Schumann region below 2000 Å. The ethylenediamine complexes showed a general absorption in the vicinity of 2600 Å. Although correlation with stability is difficult, it is generally conceded that a lower excited state is frequently associated with a shift of absorption to

longer wavelength, and from the available data this is so for the complexes investigated. Since the absorption curves lie very closely together and converge they have not been included in this work, but the absorption maxima are given in Table 4.

TABLE 4

ABSORPTION SPECTRA OF MERCURY(II) COMPLEXES IN 95 PER CENT. ETHANOL AT [10^{-5}M]

Substance	Maxima (Å)	Substance	Maxima (Å)
Cl^-	1820 (Scheibe 1929)	HgI_2·en	2610
Br^-	1900, 1995 (Scheibe 1929)	$HgCl_2$·phen	2255, 2720
I^-	1940 (Scheibe 1929)	$HgBr_2$·phen	2250, 2720
Phenan	2250, 2600	HgI_2·phen	2250, 2710
Dipy	2370, 2810 (Gillam, Hey, and Lambert 1941)	$HgCl_2$·dipy	2370, 2900
$HgCl_2$·en	2550	$HgBr_2$·dipy	2370, 2905
$HgBr_2$·en	2580	HgI_2·dipy	2375, 2910

II. EXPERIMENTAL

The conductivity measurements were carried out according to the method outlined in earlier papers, the purified nitrobenzene being the same as that used previously. The nitromethane of A.R. quality was purified by drying over anhydrous calcium sulphate and retaining the fraction boiling at 101–101·5 °C. The methanol was purified by the method of Morton and Mark, by treating methanol (500 ml) with furfural (25 ml) and 10% sodium hydroxide (60 ml), and refluxing for 8 hr. After distilling it was dried by heating with magnesium activated by iodine, refluxed and redistilled. A slight correction was made for the low conductivity of the solvent. Ethylene-dibromide (B.D.H. Laboratory Standard) was distilled and the fraction boiling between 131 and 132 °C retained. Ebulliometric measurements were made in this solvent using the Sucharda-Bobranski apparatus.

The solubility of the complexes in dilute strong acid, due to decomposition, enabled the anions to be estimated as follows: sulphate (as $BaSO_4$), nitrite and oxalate (with MnO_4^-), and halide (as AgX). The mercury was estimated by the iodide ion (Seamon's method). The measurements of absorption spectra were made with a Beckmann quartz spectrophotometer, model DU, within the wavelength range of 2180 to 3500 Å.

(*a*) *Dichloromonoethylenediamine Mercury(II)*.—Mercuric chloride (2·72 g; 10mM) in 50% ethanol (50 ml) was treated with ethylenediamine (0·60 g; 10 mM) with stirring. The white precipitate which formed was separated by centrifuging, washed with ethanol, then with diethyl ether, and dried in a vacuum desiccator over calcium chloride (yield 3·3 g) (Found: C, 7·5; H, 2·5; Cl, 21·5%. Calc. for $C_2H_8N_2Cl_2Hg$: C, 7·2; H, 2·4; Cl, 21·4%).

(*b*) *Dibromomonoethylenediamine Mercury(II)*.—The above procedure was repeated using mercuric bromide (3·60 g; 10 mM), yield 4·1 g (Found: C, 5·7; H, 1·9; Br, 38·5; Hg, 47·5%. Calc. for $C_2H_8N_2Br_2Hg$: C, 5·7; H, 1·9; Br, 38·0; Hg, 47·7%).

(*c*) *Di-iodomonoethylenediamine Mercury(II)*.—The latter procedure was repeated using a solution of mercuric iodide (4·55 g; 10 mM) in sufficient potassium iodide, water (10 ml), and ethanol (15 ml), yield 5·14 g (Found: C, 4·4; H, 1·5; I, 50·0%. Calc. for $C_2H_8N_2I_2Hg$: C, 4·7; H, 1·6; I, 49·5%).

(*d*) *Dichloromonophenanthroline Mercury(II)*.—Mercuric chloride (0·272 g; 1 mM) in 30% ethanol (5 ml) was allowed to react with 1,10-phenanthroline monohydrate (0·198 g; 1 mM) in ethanol (2 ml) with stirring. The white precipitate which settled rapidly was removed by centrifuging, redissolved in ethanol, and reprecipitated with diethyl ether and dried in a vacuum desiccator over calcium chloride, yield 0·43 g (Found: C, 32·1; H, 1·8; Cl, 15·9%. Calc. for $C_{12}H_8N_2Cl_2Hg$: C, 31·9; H, 1·8; Cl, 15·7%).

(*e*) *Dibromomonophenanthroline Mercury(II)*.—The procedure outlined in (*d*) was repeated using mercuric bromide (0·36 g; 1 mM), a fine white powder resulting, yield 0·52 g (Found: C, 26·4; H, 1·5; Br, 29·3; Hg, 37·1%. Calc. for $C_{12}H_8N_2Br_2Hg$: C, 26·7; H, 1·5; Br, 29·6; Hg, 37·1%).

(*f*) *Di-iodomonophenanthroline Mercury(II)*.—The procedure described in (*d*) was repeated using mercuric iodide (0·46 g) in sufficient potassium iodide and 50% ethanol (6 ml). A very pale yellow microcrystalline powder resulted, yield 0·63 g (Found: C, 22·9; H, 1·4; I, 40·4%. Calc. for $C_{12}H_8N_2I_2Hg$: C, 22·7; H, 1·3; I, 40·0%).

(*g*) *Dichloromonodipyridyl Mercury(II)*.—Procedure (*d*) was repeated using dipyridyl (0·156 g; 1 mM) in lieu of phenanthroline. A white microcrystalline powder resulted, yield 0·40 g (Found: C, 28·0; H, 1·8; Cl, 16·5%. Calc. for $C_{10}H_8N_2Cl_2Hg$: C, 28·1; H, 1·9; Cl, 16·6%).

(*h*) *Dibromomonodipyridyl Mercury(II)*.—Procedure (*e*) was repeated using dipyridyl (0·156 g; 1 mM). A white powder was obtained, yield 0·50 g (Found: C, 23·0; H, 1·4; Br, 31·0%. Calc. for $C_{10}H_8N_2Br_2Hg$: C, 23·3; H, 1·6; Br, 30·9%).

(*i*) *Di-iodomonodipyridyl Mercury(II)*.—Procedure (*f*) was repeated using dipyridyl (0·156 g; 1 mM). A pale yellow powder resulted, yield 0·60 g (Found: C, 19·8; H, 1·3; I, 41·0%. Calc. for $C_{10}H_8N_2I_2Hg$: C, 19·7; H, 1·3; I, 41·5%).

(*j*) *Bisphenanthroline Mercury(II) Nitrate*.—A solution of mercuric nitrate (0·33 g; 1 mM) in water (5 ml) was treated with drops of nitric acid and allowed to react with phenanthroline monohydrate (0·60 g; 3 mM) in ethanol (2 ml). The white crystalline precipitate which settled out on standing was recrystallized from dilute ethanol and dried in a vacuum desiccator over calcium chloride, yield 0·67 g (Found: C, 41·7; H, 2·2; Hg, 29·4%. Calc. for $C_{24}H_{16}N_6O_6Hg$: C, 42·0; H, 2·3; Hg, 29·2%).

(*k*) *Bisdipyridyl Mercury(II) Perchlorate*.—Procedure (*j*) was repeated using dipyridyl (0·47 g) in lieu of phenanthroline and adding perchloric acid (1·0 ml) since the nitrate did not readily precipitate, yield 0·71 g (Found: C, 33·3; H, 2·5; Hg, 27·9%. Calc. for $C_{20}H_{16}N_4O_8Cl_2Hg$: C, 33·7; H, 2·3; Hg, 28·2%).

(*l*) *Dinitromonoethylenediamine Mercury(II)*.—Mercuric nitrate (0·98 g; 3 mM) in water (5 ml) was treated with drops of nitric acid and diluted to 50 ml with water, and sodium nitrite (8 g) added before the addition of ethylenediamine (0·18 g; 3 mM) in water (2 ml). The white precipitate which formed after evaporating to half-volume was separated by centrifuging, washed with a little ethanol, then with diethyl ether, and dried in a vacuum desiccator, yield 1·01 g (Found: NO_3, 25·9; Hg, 56·5%. Calc. for $C_2H_8N_4O_6Hg$: NO_3, 26·1; Hg, 56·9%).

(*m*) *Dinitromonophenanthroline Mercury(II)*.—Procedure (*l*) was repeated using phenanthroline hydrate (0·59 g) in ethanol (2 ml) instead of ethylenediamine. A white microcrystalline powder was formed almost immediately, yield 1·40 g (Found: NO_3, 19·2; Hg, 42·5%. Calc. for $C_{12}H_8N_4O_6Hg$: NO_3, 19·5; Hg, 42·5%).

(*n*) *Sulphatomonoethylenediamine Mercury(II)*.—Mercuric sulphate (1·0 g) was dissolved in water (40 ml) and drops of dilute sulphuric acid added to suppress hydrolysis before the addition of ethylenediamine (0·18 g) in water (2 ml). Colourless plates settled out after stirring, and these were separated, washed with ethanol and then with ether before drying in a vacuum desiccator over calcium chloride, yield 0·98 g (Found: SO_4, 27·3; Hg, 56·4%. Calc. for $C_2H_8N_2SO_4Hg$: SO_4, 26·9; Hg, 56·3%).

(*o*) *Sulphatomonophenanthroline Mercury(II)*.—Procedure (*n*) was repeated using phenanthroline hydrate (0·59 g) in lieu of ethylenediamine, yield 1·40 g (Found: SO_4, 20·3; Hg, 42·0%. Calc. for $C_{12}H_8N_2SO_4Hg$: SO_4, 20·2; Hg, 42·1%).

(*p*) *Oxalatomonoethylenediamine Mercury(II)*.—Mercuric nitrate (1·0 g) in water (40 ml) was treated with a mixture of ammonium oxalate (1 g) and ethylenediamine (0·18 g) in water (10 ml). The white powdery precipitate which formed was treated as in procedure (*n*), yield 1·02 g (Found: C_2O_4, 25·0; Hg, 57·7%. Calc. for $C_4H_8N_2O_4Hg$: C_2O_4, 25·2; Hg, 57·6%).

(*q*) *Oxalatomonophenanthroline Mercury(II)*.—Procedure (*p*) was repeated using phenanthroline hydrate (0·59 g) in ethanol (5 ml) in lieu of ethylenediamine, yield 1·40 g (Found: C_2O_4, 18·6; Hg, 42·6%. Calc. for $C_{14}H_8N_2O_4Hg$: C_2O_4, 18·8; Hg, 42·9%).

III. Acknowledgment

The author is grateful to Dr. E. Challen of the Microanalytical Laboratory for the carbon and hydrogen analyses.

IV. References

Gillam, A. E., Hey, D. H., and Lambert, A. (1941).—*J. Chem. Soc.* **1941**: 364.
Infeldt, G., and Sillén, L. G. (1946).—*Svensk Kem. Tidskr.* **58**: 104.
Ley, H., and Kissel, H. (1899).—*Ber. dtsch. chem. Ges.* **32**: 1361.
Morgan, G. T., and Burstall, F. H. (1930).—*J. Chem. Soc.* **1930**: 2594.
Nyman, C. J., Roe, D. K., and Mason, D. B. (1955).—*J. Amer. Chem. Soc.* **77**: 4191.
O'Brien, T. D. (1948).—*J. Amer. Chem. Soc.* **70**: 2771.
Pfeiffer, P., and Christeleit, W. (1938).—*Z. anorg. Chem.* **239**: 133.
Pfeiffer, P., Schmitz, E., and Bohm, A. (1952).—*Z. anorg. Chem.* **270**: 287.
Pfeiffer, P., and Werdelmann, Br. (1950).—*Z. anorg. Chem.* **261**: 197.
Roloff, M. (1894).—*Z. phys. Chem.* **13**: 327.
Rosenheim, A., and Oppenheim, K. (1901).—*Z. anorg. Chem.* **28**: 171.
Schaefer, H. (1905).—*Z. anorg. Chem.* **45**: 299.
Scheibe, G. (1929).—*Z. phys. Chem.* (Abt. B) **5**: 355.
Straumaris, M., and Circulis, A. (1936).—*Z. anorg. Chem.* **230**: 65.
Watters, J. T., and Mason, J. G. (1956).—*J. Amer. Chem. Soc.* **78**: 285.
Weitz, E., Blasberg, K., and Wernicke, E. (1930).—*Z. anorg. Chem.* **188**: 344.

A XANTHENE POLYMER WITH SEMICONDUCTING PROPERTIES

By R. McNeill* and D. E. Weiss*

[*Manuscript received June* 12, 1959]

Summary

A series of xanthene polymers has been prepared as the first stage of a study of the potentialities of organic semiconductors for the synthesis of model enzymes, and for the development of ion-exchange adsorbents capable of direct electrical regeneration. The polymer is composed of condensed xanthene residues cross-linked with phenyl bridges. The polymers are *p*-type semiconductors and the best has a specific resistance of $7 \times 10^3\ \Omega$ cm which is less than that of crystalline organic semiconductors by a factor of at least 10^4. The conductivity is not due to the diradicals of the quinonoid resonance form of quinolphthalein, but is more probably due to a resonating carbonium cation arising from partial ionization of the xanthylium–lactone bond. The effect of polymerization conditions on the resistance of the polymer has been studied.

I. Introduction

It is becoming increasingly evident that some biological systems are capable of conducting electricity electronically rather than electrolytically. Thus the respiratory cytochrome system probably contains an electronic conductor (Geissman 1949) and *p-n* junctions may occur within the lamellar structure of the chloroplasts permitting them to function as a photocell in which oxidation and reduction reactions occur on opposite sides of the lamellae (Calvin 1955). The electronic conductivity of both these systems, together with the redox properties of the attached porphyrins or the *iso*alloxazine nucleotides, is fundamental to their functioning as the primary catalysts of respiration and photosynthesis, the vital steps in the processes of energy transformation in the living world, but unequivocal data derived from simplified models of these systems are required before a full understanding can be achieved.

The most familiar of the organic semiconductors, activated carbon, also behaves as an oxidase (reviewed by Garten and Weiss 1957*a*). Recently Kearns and Calvin (1958) have produced a *p-n* junction from organic semiconductors which produces an electric current on illumination.

These two examples thus suggest that the systematic development of new organic semiconductors to which specific functional groups can be attached could lead ultimately to the production of synthetic enzymes, modelled on their biological counterparts, which could lead to new processes of energy transformation. It has been shown also how such semiconductors could lead to a new ion-exchange process for the desalination of water (McNeill and Weiss 1959). However, technical exploitation will depend on the production of new organic

* Division of Physical Chemistry, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

semiconductors having a very much lower electrical resistance than the majority of those reported to date. Their field of application will be extended if they can be made with a high internal surface to permit the free entry of ions as in ion-exchange resins. The latter requirement presupposes a non-lamellar structure, as in activated carbon, in contrast to the lamellar structure of the systems studied to date.

This paper describes the first results of a search for such materials in these laboratories.

II. Organic Semiconductors

The need for high electronic conductivity requires a new class of polymer, since, with the exception of activated carbon, organic polymers as known today are excellent insulators. Thus, the specific resistance of a phenol–formaldehyde resin is about 10^9 to 10^{15} Ω cm whereas that of activated carbon is about 10^{-2} Ω cm. The semiconductivity of various crystals of aromatic hydrocarbons and phthalocyanine has been measured by Eley *et al.* (1953) and Eley and Parfitt (1955) and values of the order of $1{\cdot}5\times10^7$ Ω cm or higher have been observed. However, a bromine-perylene complex with an exceptionally low resistance of 8 Ω cm has been prepared by Akamatsu, Inokuchi, and Matsunaga (1956).

Unlike graphite, which has a lamellar structure and a metallic type conductivity, activated carbon is exceptional as a semiconductor since it contains non-planar aromatic structures and therefore has a high internal surface area on which its adsorbent properties depend. Although its electrical resistance is very low, it is not ideally suitable for the introduction of the functional groups required for its conversion into a more effective catalyst or ion-exchange adsorbent because of its complex and variable structure. Since it is produced by condensation and dehydrogenation reactions at high temperatures, methods have been devised in this laboratory to prepare other carbonaceous polymers at temperatures not exceeding 250 °C by analogous reactions performed with more reactive chemical raw materials and catalysts. In this way functional groups introduced with the raw materials may be retained in the final polymer. Furthermore polymers can be produced with reasonably well-defined and reproducible structures.

In the current work microporous xanthene polymers have been prepared, the best having a specific resistance of 7×10^3 Ω cm. This forms part of a wider investigation into the semiconductivity of polymers formed from dyestuffs of the triphenylmethane class. Xanthene derivatives have been studied first as these are amongst the simplest to prepare.

III. Conditions for Polymer Preparation

The simplest xanthene dyestuff, fluorescein, is obtained by heating 2 moles of resorcinol with 1 mole of phthalic anhydride to 150–200 °C in the presence of zinc chloride or sulphuric acid. The dyestuff results from substitution by the phthalic anhydride at only one of the two positions *para* to the hydroxyls of resorcinol so that the other position is left free. It is to be expected, therefore, that if only 1 mole of resorcinol were used per mole of phthalic anhydride further condensation would occur at the position *ortho* to the hydroxyl in fluorescein

to yield a linear polymer. Thus a black tar, soluble in alkali but insoluble in ethanol, is obtained under these conditions. A more insoluble polymer results if some of the phthalic anhydride is replaced by pyromellitic dianhydride which introduces cross-linkages into the structure. A completely insoluble polymer is produced if resorcinol is replaced by hydroquinone. This is most likely polymerized quinolphthalein (Fig. 1) in which linear chains are cross-linked by phenyl bridges resulting from condensation with pyromellitic dianhydride. The condensation is sterically more favourable when hydroquinone rather than resorcinol is used, as *trans* cross-links may be then introduced, whereas in the latter instance they must be *cis*. Some contamination of the polymer by anthraquinone

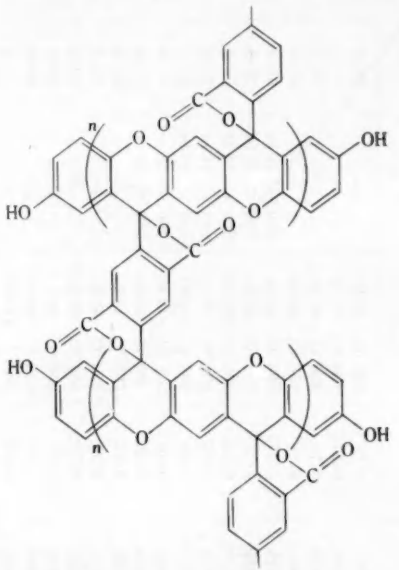


Fig. 1.—Suggested structure for the xanthene polymer.

structures is possible, depending on reaction conditions, since these are known by-products in xanthene melts. This may be minimized by using zinc chloride as the catalyst (Copisarow 1920).

A number of xanthene polymers have been prepared under different conditions which are summarized in Table 1. Only polymers that are completely insoluble in aqueous acid and alkali are reported. An attempt has been made to ascertain the influence of conditions of polymerization on the specific resistance at 25 °C of a dry plug of the polymer in an atmosphere of pure nitrogen. The resistance increases when oxygen is admitted and slowly falls on admitting nitrogen again. Details of the procedure are given in Section VIII.

A linear relationship exists between the logarithm of the resistance of the polymer at various temperatures and the reciprocal of the absolute temperature

TABLE 1

SUMMARY OF POLYMERIZATION CONDITIONS

Sample No.	Hydroquinone (mole)	Phthalic Anhydride (mole)	Pyromellitic Dianhydride (mole)	Zinc Chloride (mole)	First Heating	Second Heating	Carbon (%)	Hydrogen (%)	Oxygen (%)	Sulphate Ash (%)
75	0·1	0	0·05	0·75	200 °C, 24 hr	—	66·0	3·30	24·4	0·34
78	0·1	0·05	0·025	0·375	200 °C, 24 hr	—	70·1	3·20	25·6	<0·1
79	0·1	0·05	0·025	0·75	250 °C, 48 hr	—	70·2	3·0	25·8	<0·1
80	0·1	0	0·05	0·75	250 °C, 48 hr	—	63·8	3·5	30·4	0·58
83	0·1	0·05	0·025	0·375	200 °C, 48 hr	250 °C, 24 hr	65·8	3·6	29·2	0·32
84	0·1	0·05	0·025	0·75	200 °C, 24 hr	300 °C, 24 hr	73·0	2·98	23·2	0·27
85	0·1	0·075	0·0125	0·75	200 °C, 24 hr	250 °C, 24 hr	73·2	2·83	23·1	0·29
87	0·1	0·05	0·025	0·75	200 °C, 48 hr	250 °C, 24 hr	62·2	3·67	31·7	0·21
88	0·1	0·075	0·0125	0·75	200 °C, 48 hr	250 °C, 24 hr	62·1	4·33	31·4	0·20
89	0·1	0·05	0·025	0·75	200 °C, 48 hr	300 °C, 24 hr	71·9	2·96	24·8	<0·1
101	0·3	0·15	0·075	2·25	200 °C, 48 hr	250 °C, 24 hr	72·8	2·95	23·9	0·40
104	0·1	0·05	0·025	0·75	250 °C, 48 hr	—	69·0	3·16	26·7	0·20
109	0·1	0·05	0·025	0·75	250 °C, 66 hr	—	70·0	3·22	25·6	0·20
110	0·1	0·05	0·025	0·75	250 °C, 66 hr*	—	69·5	3·11	26·9	0·14
111	0·1	0·05	0·025	0·75	250 °C, 96 hr	—	70·0	3·32	24·9	0·25
112	0·1	0·05	0·025	0·75	250 °C, 96 hr*	—	69·0	2·98	26·9	0·12
113	0·1	0·05	0·025	0·75	250 °C, 120 hr	—	71·3	3·49	25·0	0·30
114	0·1	0·05	0·025	0·75	250 °C, 120 hr*	—	68·1	2·93	27·1	0·31
117	0·1	0·05	0·025	0·75	200 °C, 48 hr	250 °C, 24 hr	57·8	4·76	33·6	<0·1
118	0·1	0·05	0·025	0·75	250 °C, 24 hr	400 °C, 24 hr	87·1	2·49	8·6	0·60
119	0·1	0·075	0·0375	0·75	200 °C, 48 hr	250 °C, 24 hr	70·5	3·14	25·7	0·30
121	0·1	0·025	0·0125	0·75	200 °C, 48 hr	250 °C, 24 hr	73·8	3·13	22·1	0·30

* Sample prepared in an atmosphere of air. All other samples prepared in nitrogen.

(Fig. 2). The electronic nature of the conductivity is suggested by the lack of polarization observed during the resistance measurements since a steady current results immediately on applying the potential. Thus it behaves as a typical electronic semiconductor (Wilson 1939).

The resistance of the polymer is very sensitive to minor differences in the conditions of polymerization and particularly to time of exposure to air. It has therefore not been possible to reproduce exactly the resistance of polymers

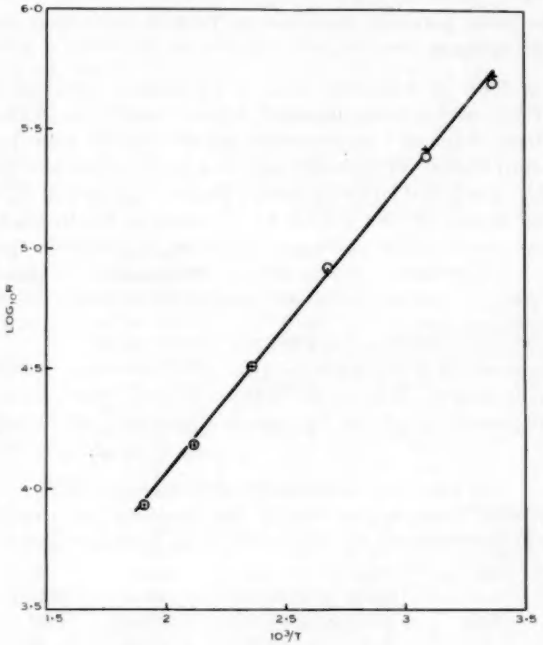


Fig. 2.—The resistance of polymer No. 101 as a function of temperature. × Ascending conditions. ○ Descending conditions.

produced under seemingly identical conditions although temperature controllers were used to ensure constancy of temperature and the polymers from No. 104 onwards were stored under nitrogen. This is illustrated by the following series :

Number ..	87	117	101
R (kΩ cm)	20	7	72
	(small batch)	(small batch)	(large batch)

Hence valid conclusions may be drawn from the following experiments only when very large differences are observed. Nevertheless they serve to establish the more important variables in the synthesis.

(*a*) *Effect of Air*

The resistance of the polymer is lower, and it contains a little less oxygen when prepared in an atmosphere of nitrogen rather than air :*

No.	Time of Heating (hr)	R_{N_2} (kΩ cm)	R_{O_2} (kΩ cm)	O_{N_2} (%)	O_{O_2} (%)
109–110	66	70	147	25·6	26·9
111–112	96	82	186	24·9	26·9
113–114	120	1290	5750	25·0	27·1

Hence all the other polymers described in Table 1 have been prepared in an atmosphere of nitrogen.

(*b*) *Ratio of Phthalic Anhydride (PA) to Pyromellitic Dianhydride (PMA)*

If only PA is used a tar is produced which is soluble in alkali and which is probably a linear polymer. An insoluble polymer results when PMA is added. If the total anhydride–hydroquinone ratio is kept constant and the proportion of PA to PMA varied, the following results suggest that at 250 °C the resistance of the polymer is not affected greatly by the ratio of PA to PMA :

No.	PA (mole)	PMA (mole)	Time and Temperature	*R* (kΩ cm)
79	0·05	0·025	250 °C, 48 hr	35
80	0	0·05	250 °C, 48 hr	35
87	0·05	0·025	200 °C, 48 hr	
			250 °C, 24 hr	20
117	0·05	0·025	200 °C, 48 hr	
			250 °C, 24 hr	7
88	0·075	0·0125	200 °C, 48 hr	30
			250 °C, 24 hr	

(*c*) *Ratio of Anhydride to Hydroquinone (HQ)*

It is desirable to use approximately the theoretical ratio or a small excess of anhydride to hydroquinone as a big excess of the latter increases the resistance :

No.	HQ (mole)	PA (mole)	PMA (mole)	*R* (kΩ cm)
121	0·1	0·025	0·0125	168
87	0·1	0·05	0·025	20
119	0·1	0·075	0·0375	23

(*d*) *Catalyst Concentration*

A much larger concentration of zinc chloride has been used than is usual in the preparation of xanthene dyestuffs since molten zinc chloride is an effective solvent for the components of the polymerization above 200 °C. The following experiments show the effect of the ratio of zinc chloride to hydroquinone :

No.	Zinc Chloride (mole)	Hydroquinone (mole)	*R* (kΩ cm)
83	0·375	0·1	57
87	0·75	0·1	21

* For convenience, only the relevant variables will be quoted in this Section. Reference to Table 1 provides details of the conditions of polymerization.

(*e*) *Time and Temperature*

Excessive heating at 250 °C is undesirable and the oxygen content of the polymer is gradually reduced during heating:

No.	Time and Temperature	*R* (kΩ cm)	O (%)
104	250 °C, 48 hr	90·5	26·7
109	250 °C, 66 hr	70	25·6
111	250 °C, 96 hr	82	24·9
113	250 °C, 120 hr	1290	25·0

It may also be desirable to heat the reaction mass at 200 °C for some time before finishing at 250 °C:

No.	PMA (mole)	Temperature	*R* (kΩ cm)
85	0·0125	200 °C, 24 hr; 250 °C, 24 hr	69·4
88	0·0125	200 °C, 48 hr; 250 °C, 24 hr	31·2
87	0·025	200 °C, 48 hr; 250 °C, 24 hr	20·7

Much more work will be required to establish the optimum combination of time and temperature but a cycle of 48 hr at 200 °C followed by 24 hr at 250 °C has been selected tentatively as most desirable.

(*f*) *Temperature*

The optimum temperature for the polymerization is in the region of 250 °C. At higher temperatures decarboxylation occurs (see Section IV), whereas at lower temperatures the reaction is slower and non-homogeneous since the mass has not melted.

No.	Temperature	*R* (kΩ cm)
89	200 °C, 48 hr; 300 °C, 24 hr	72·5
87	200 °C, 48 hr; 250 °C, 24 hr	20
84	200 °C, 24 hr; 300 °C, 24 hr	172
83*	200 °C, 48 hr; 250 °C, 24 hr	57
118	250 °C, 24 hr; 400 °C, 24 hr	20,000

(*g*) *Condensation Catalysts*

Neither polyphosphoric acid nor concentrated sulphuric acid were satisfactory condensation catalysts for the polymerization. The former did not yield insoluble polymers and these could only be obtained with sulphuric acid at about 150 °C where it is known that migration of the lactone ring occurs to yield ceroxenols (Decker 1906). Some sulphonation also occurred and higher temperatures produced a soluble product. Because these polymers have a very high resistance, sulphuric acid was abandoned in favour of zinc chloride. One polymer produced by heating 0·1 mole HQ, 0·05 mole PA, 0·025 mole PMA with 7·3 moles of concentrated sulphuric acid for 48 hr at 150 °C had a specific resistance exceeding 20,000,000 Ω cm and contained 4·35 per cent. sulphur.

* Sample 83 was prepared with only half the amount of zinc chloride used in Nos. 84, 87, 89, and 118.

IV. The Influence of Structure on Resistance

A large batch of product No. 101 was produced and a variety of derivatives was prepared in the following manner.

(i) *Heat Treatment.*—On heating the polymer in a stream of nitrogen at 250 °C a red sublimate is produced. Heating for long periods increases the resistance :

No.	Time (hr)	R (kΩ cm)
101	6	71·6
	48	690

Portions of the polymer were also heated at different temperatures for 24 hr in a stream of nitrogen. As shown in Table 2, this resulted in a reduction of the oxygen content of the polymer with increase in temperature. The resistance rose to a maximum in the region of 500 °C and then fell sharply. The sudden fall in resistance recalls that which occurs when sugar char is heated to about 650 °C.

Table 2

Effect of Heat Treatment on Product No. 101

Temp. (°C)	O (%)	R (Ω cm)		Total-CH_3O (m-equiv/g)	NaOH Adsorption (m-equiv/g)	N Lactone* (m-equiv/g)	Phenolic OH† (m-equiv/g)	F Lactone‡ (m-equiv/g)
		Un-methylated	Methylated					
25	23·9	$7·2\times10^4$	$7·6\times10^6$	1·64	4·03	2·39	1·18	0·46
400	17·6	$18·2\times10^4$	$3·1\times10^6$	0·20	1·01	0·80	0·14	0·06
500	12·2	$28·3\times10^4$	$2·4\times10^6$	0·100	—	—	0·04	0·06
550	8·2	$2·5\times10^3$	—	—	—	—	—	—
650	4·4	2·3	3·9	0·06	0·22	0·2	0·02	Nil
800	3·7	0·3	0·2	0·06	0·27	0·2	0·02	Nil

* N lactone = (NaOH adsorption)—(total methoxyl).

† Phenolic hydroxyl = non-hydrolysable methoxyl.

‡ F lactone = (total methoxyl)—(non-hydrolysable methoxyl).

(ii) *Methylation.*—A number of polymers were methylated with diazomethane in ether according to the procedure of Hofmann and Ohlerich (1950). The resistance rose sharply after methylation :

No.	R (kΩ cm)	
	Unmethylated	Methylated
80	35·6	7500
88	31·2	6300
101	71·6	7600

The polymers heated to various temperatures up to 800 °C were also methylated (Table 2). Methylation increased the resistance of the polymers prepared at temperatures up to 500 °C but did not appreciably affect the resistance of the polymers heated to 650 and 800 °C.

(iii) *Effect of Alkali.*—Polymer No. 101 adsorbed 4·03 m-equiv/g of sodium hydroxide when shaken with excess 0·2N alkali for 60 hr. Portions of the polymer were treated with smaller increments of alkali so that the acidity of the polymer was never fully neutralized. The treated polymers were then dried, and the resistance was measured at 25 °C after drying in a stream of nitrogen in the conductivity cell at 150 °C. The alkali increased the resistance of the polymer considerably.

NaOH (m-equiv/g)	R (kΩ cm)	NaOH (m-equiv/g)	R (kΩ cm)
0	72	0·50	3120
0·25	1390	1·125	5800

V. Structure of the Polymer

Since the polymerization conditions are similar to those for the synthesis of quinolphthalein (Meyer and Spengler 1903), it is reasonable to postulate that the polymer is composed essentially of condensed strings of quinolphthalein cross-linked with phenyl bridges as shown in Figure 1.

Some confirmation of this structure may be inferred by a procedure of functional group analysis that has been developed for carbon black (Garten, Weiss, and Willis 1957*b*) which establishes the presence of the expected functional groups. A lactone associated with a phenolic hydroxyl group will open to form a quinone with alkali as does that of the parent quinolphthalein (V, Fig. 3). This lactone, which will be referred to as an F lactone, has been identified in carbon black. Lactones occupying intermediate positions along the chain adjacent to several xanthene rings will not be able to form quinones and have been termed N lactones. Both F and N lactones react with alkali, but only F lactones are methylated with diazomethane. Hence the difference between the total acidity of the polymer as determined by alkali adsorption and by methylation indicates the concentration of N lactones in the polymer (Table 2). The difference between the methoxyl content of the polymer after methylation with diazomethane and after the polymer has been hydrolysed with boiling acid indicates the concentration of F lactones and free carboxyls. The concentration of the non-hydrolysable methoxyl groups is a measure of the phenolic hydroxyls in the polymer.

A titration curve of the polymer, prepared by a method described elsewhere (Garten, Weiss, and Willis 1957*b*), conforms with that of a very weak acid and is similar to that of an ink carbon black, so that only a very low concentration of free carboxyl groups is present. It will be assumed, therefore, that the methoxyl groups hydrolysed by acid indicate the concentration of F lactones. The results of such an analysis on polymer No. 101 are presented in Table 2.

Table 2 shows that the N lactones are more stable than the F lactones at 400 °C and that the concentration of the latter is negligible in the polymer heated beyond this temperature. This follows since decarboxylation of an F lactone produces a stable quinone, whereas that of a N lactone produces an unstable diradical and is consequently more difficult.

For product No. 101 there are five N lactones for every F lactone and two N lactones for every phenolic hydroxyl. The observed average value of one phenolic hydroxyl per two N lactones indicates that ring closure to xanthene units is far from complete. Completion of this ring closure could account for the low phenolic content of the polymer after heating to 400 °C.

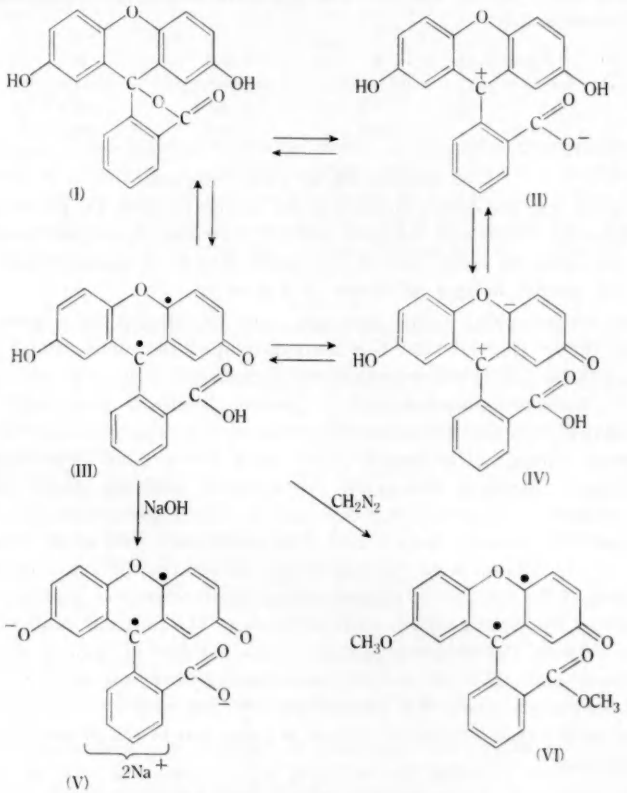


Fig. 3.—Reactions of quinolphthalein.

It is probable that the very high resistance of the polymer prepared with sulphuric acid as a catalyst is due to anthraquinones whose formation is favoured by this catalyst (Copisarow 1920). Ceroxenols rather than lactones would also tend to be produced under these conditions.

VI. Discussion

A xanthene dyestuff, such as quinolphthalein for example, may be regarded as an inner salt (Fig. 3) between the xanthylium cation and the carboxylate anion (Ramette and Sandell 1956). Only slight dissociation can occur in the

polymer since both are weak electrolytes. The polymer could also exist in tautomeric equilibrium with a low concentration of the unstable diradical III.* Since the exceptionally low activation energy of activated carbon and crystalline $\alpha\alpha$-diphenyl β-picryl hydrazyl is probably due to their free radical structures (Mrozowski 1952, 1953*a*, 1953*b*; Eley 1955) it might be thought that diradicals III in the xanthene polymer are responsible for its low conductivity.

At room temperature these might partially pair as in IV to yield migrating charges in which case treatment with alkali or diazomethane should lower the resistance since this treatment would open the lactone ring as in V and VI and enormously increase the concentration of the conducting species. The converse

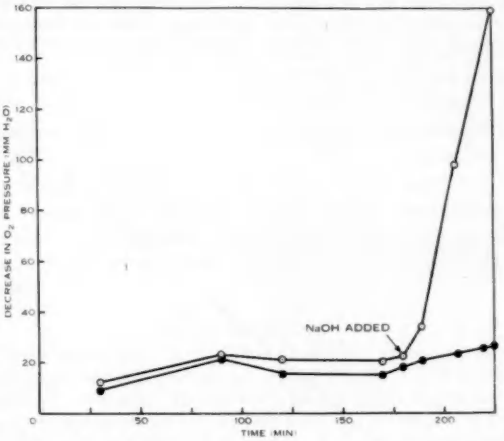


Fig. 4.—Absorption of oxygen by polymer No. 117.
● Blank in water. ○ Sample to which alkali was added.

actually occurs. The production of diradicals is consistent with the rapid adsorption of oxygen by the polymer upon the addition of alkali (Fig. 4). Such an absorption would bind the electrons of the diradical and prevent their contribution to the conductivity of the polymer. The increased resistance of the polymer after prolonged standing in air may be due to this factor. Some of the oxygen adsorption could be due to oxidation of the phenolic groups of the polymer but this alone would not account for the observation that the electrical resistance of a polymer after exposure to oxygen could be restored to about its former value by the prolonged passage of a stream of nitrogen through a plug of the polymer.

* An alkaline solution of quinolphthalein is coloured intensely violet but, unlike the quinonoid form of phenolphthalein, is unstable towards oxygen. A brown precipitate was obtained on bubbling oxygen through the solution overnight. Diradicals could explain this. The cause of the colour change has been the subject of much speculation and has been reviewed by Hewitt (1922).

Decarboxylation of F lactone groups will also result in the formation of a quinonoid diradical structure and as has been observed, prolonged heating between 250 and 500 °C does result in partial decarboxylation and increases the resistance of the polymer. The low resistance of the polymer heated to 800 °C, which is not appreciably altered by methylation, is undoubtedly due to a gross rearrangement of the polymer lattice at these temperatures similar to that which occurs with a sugar char so that the mechanism of the conductivity of these polymers is likely to be quite different from those prepared at lower temperatures. Thus thermobalance measurements on polymer No. 117 show that the maximum rate of loss in weight during pyrolysis in nitrogen occurs at 660 °C. It may be concluded therefore that the presence of quinonoid diradical structures is not the major factor responsible for the electrical conductivity of the polymer.

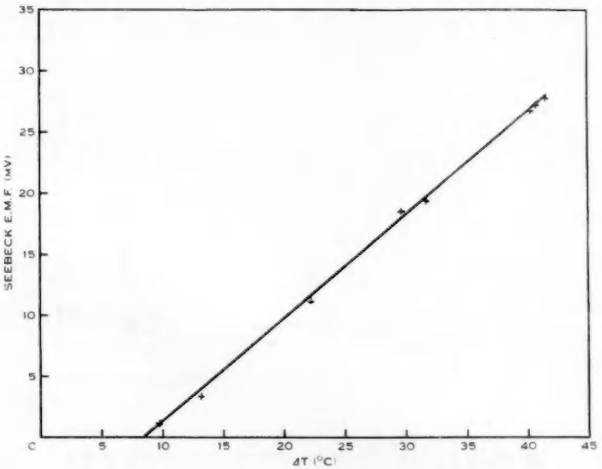


Fig. 5.—Thermoelectric properties of polymer No. 117.

The presence of the dissociated structure II, even in low concentrations, could account for the conductivity of the polymer, since migration of the positive charge across the central carbon atom of the xanthene is possible and would be facilitated by the neighbouring negative charge of the carboxyl group. On this basis, the polymer should show a *p*-type semiconductivity in accordance with an observed positive thermoelectric power of 900/μV/deg (Fig. 5) (Wilson 1939). This structure disappears when the lactone ring is opened by methylation or by the formation of a sodium salt.

It has not been possible to remove completely the ash constituents of the polymer, but the profound influence of alkali and diazomethane on its conductivity renders it improbable that the ash makes anything but a minor contribution, if at all, to the conductivity.

The large increase in resistance produced by methylation, or by the formation of a sodium salt, suggests that the semiconductivity is associated with the lactone

ring and not with processes involving the passage of an electron from one polymer molecule to another. This is in marked contrast to most of the organic semiconductors examined to date since the latter process appears to be the cause of the semiconductivity of aromatic crystals. Thus a pure aromatic ring should show superconductivity, if ohmic contact to a single aromatic molecule could be made (London 1937). Hence the xanthene polymers may be regarded as impurity *p*-type semiconductors in which the carboxyl ion is the " impurity " electron acceptor.

VII. Conclusions

The above results show that it is possible to synthesize, by very simple means, organic polymers with the properties of *p*-type semiconductors. As organic semiconductors, their resistance is exceptionally low compared with that of the organic crystals studied previously whose resistance exceeds $1 \cdot 5 \times 10^7$ Ω cm. However, their resistance is high compared with activated carbon. The study also shows how polymers, similar in many ways to activated carbon, may be produced by the methods of synthetic organic chemistry rather than by pyrolysis, and how a simple structural change, such as the formation of an ester or a salt, can result in a major change in the electrical resistance of the polymer. These considerations suggest that in the future, further knowledge of the organic chemistry of such polymers must result ultimately in the production of a wide variety of polymers with even lower resistances and individually synthesized for specific purposes.

VIII. Experimental

(*a*) *Method of Polymerization.*—In a typical experiment hydroquinone, phthalic anhydride, pyromellitic dianhydride, and zinc chloride are ground together to a fine powder and placed in a flask fitted with an air condenser. The system is purged with nitrogen and the top of the condenser is then connected to a rubber bladder also filled with nitrogen. The flask is placed in an air-bath whose temperature is controlled to within ±3 °C. When the specified reaction time is completed the mass is ground and extracted with acetone until the filtrate is colourless and does not give a precipitate upon the addition of dilute aqueous acid. It is then washed with 1N hydrochloric acid until the filtrate fails to give a test for zinc with dithiozone. The washing is completed with water until the filtrate is free of chloride ions detectable with silver nitrate.

(*b*) *Electrical Conductivity.*—Before measuring the electrical conductivity, the polymer is ground and sieved to produce a fraction having a particle size of +150 to −100 mesh and is then heated in a stream of nitrogen at 250 °C for 6 hr. This eliminates a red sublimate which, if released in the conductivity cell, results in erratic results. The sample is stored under nitrogen until required. It is then placed in a tube of " Alundum " and compressed between brass electrodes at a pressure of 100 lb in^2. Higher pressures resulted in only a small reduction of the resistance. The plug is dried for 5 hr at 150 °C in a current of nitrogen, after which it is cooled and the resistance measured. The nitrogen stream is not stopped until the measurements are complete. It has been found that resistance measurements made with an A.C. bridge as compared with a D.C. bridge give values only a little lower but the values reported are those measured by the direct current method. The results, expressed as specific resistances, are accurate to within ±10% and are the average of duplicates.

(*c*) *Methylation.*—Approximately 1 g of oven-dried polymer is treated for a minimum of 60 hr with a solution of diazomethane (approximately 2% by wt.) in absolute ether. The diazomethane is added at intervals of about 1 hr on the first day and about 4 hr on the second day. A single treatment is used on the third day. The treated sample is stored in a refrigerator (about 4 °C) except when the diazomethane solution is being added. The flasks are fitted with a calcium

chloride drying tube. At the end of the treatment the polymer is filtered from the solution, which still retains excess diazomethane. The polymer is then dried.

(*d*) *Thermoelectric Power.*—A plug of the polymer is clamped between two pieces of platinum foil supported by backing blocks, one of which is heated electrically and the other cooled by a self-induced convection current of air. The potential between the electrodes is measured with an electrometer for temperature differences of up to 40 °C from ambient temperature.

(*e*) *Oxygen Absorption.*—Samples (100 mg) of product No. 117 are placed in two Warburg flasks, 3·0 ml of distilled water is added to the carbon in the main compartments, and 0·2 ml of 2·0N sodium hydroxide is added to the side arm of one flask. The central compartment of both flasks contains 0·2 ml of 4% potassium hydroxide to absorb carbon dioxide. The flasks are shaken in a Warburg apparatus at 30±0·02 °C. The rate of absorption of oxygen is measured for 2½ hr, after which the alkali in the side arm of one of the flasks is tipped onto the polymer and thoroughly mixed. A strong absorption of oxygen follows immediately.

IX. Acknowledgments

The authors are indebted to Mr. J. Morrison for his technical assistance and to Mrs. J. Wardlaw for performing the methylation experiments. We are also most grateful to the following for their generous assistance with chemicals during the initial exploratory stages: Professor W. Davies of the Chemistry Department, University of Melbourne, Shell Chemical (Aust.) Pty. Ltd., Imperial Chemical Industries of Australia and New Zealand Ltd., and Henry H. York & Co. Pty. Ltd.

X. References

Akamatsu, H., Inokuchi, H., and Matsunaga, Y. (1956).—*Bull. Chem. Soc. Japan* **29**: 213.

Calvin, M. (1955).—Proc. 3rd Int. Congr. Biochem. p. 225.

Cardrew, M. H., and Eley, D. D. (1959).—Paper presented to the Faraday Soc. Discussion "Energy Transfer with Special Reference to Biological Systems".

Copisarow, M. (1920).—*J. Chem. Soc.* **117**: 209.

Decker, H. (1906).—*Liebigs Ann.* **348**: 2W.

Eley, D. D., and Parfitt, G. D. (1955).—*Trans. Faraday Soc.* **51**: 1529.

Eley, D. D., Parfitt, G. D., Perry, M. J., and Taysum, D. H. (1953).—*Trans. Faraday Soc.* **49**: 79.

Garten, V. A., and Weiss, D. E. (1957*a*).—*Rev. Pure Appl. Chem.* **7**: 116.

Garten, V. A., Weiss, D. E., and Willis, J. B. (1957*b*).—*Aust. J. Chem.* **10**: 295.

Geissman, T. A. (1949).—*Quart. Rev. Biol.* **24**: 309.

Hewitt, J. T. (1922).—"Dyestuffs Derived from Pyridine, Quinoline, Acridine, and Xanthene." Ch. 13. (Longmans Green: London.)

Hofmann, U., and Ohlerich, G. (1950).—*Angew. Chem.* **62**: 16.

Kearns, D., and Calvin, M. (1958).—*J. Chem. Phys.* **29**: 950.

London, F. (1937).—*J. Phys. Lond.* **8**: 397.

McNeill, R., and Weiss, D. E. (1959).—Paper presented to the 4th Biennial Carbon Conference, Buffalo, U.S.A., June 1959.

Meyer, R., and Spengler, O. (1903).—*Ber. dtsch. chem. Ges.* **36**: 2949.

Mrozowski, S. (1952).—*Phys. Rev. Lond.* **85**: 609.

Mrozowski, S. (1953).—*Phys. Rev. Lond.* **86**: 1056.

Ramette, R. W., and Sandell, E. B. (1956).—*J. Amer. Chem. Soc.* **78**: 4872.

Wilson, A. H. (1939).—"Semiconductors and Metals." (Cambridge Univ. Press.)

A METHOD FOR THE DETERMINATION OF THE STRUCTURE OF SATURATED BRANCHED-CHAIN FATTY ACIDS

By K. E. MURRAY*

[*Manuscript received July* 20, 1959]

Summary

A method is described for the determination of the structure of branched-chain fatty acids. It is found that the carbon chain of methyl-branched acids can be readily degraded by potassium permanganate in acetone, to give a series of acids of decreasing carbon number. Where a branch occurs the acid series is interrupted and a methyl ketone, of the same carbon number as a missing acid, is produced. Gas chromatographic examination of the ketone(s) and esterified acids gives clear evidence for the location of the branch(es).

The method is illustrated by application to tuberculostearic acid (10-methyloctadecanoic acid) and C_{27}-phthianoic acid (2,4,6-trimethyltetracosanoic acid). It appears also able to decide the location of the ring in *cyclo*propane fatty acids.

I. INTRODUCTION

In the investigation of the structure of saturated branched-chain fatty acids, many of which have been isolated from natural sources in recent years, the exact location of the branched methyl groups has presented considerable difficulty. Physical determinations such as melting point, boiling point of the methyl ester, optical rotation, X-ray crystal spacings, and the infra-red spectrum, along with microanalysis for carbon-methyl, can give evidence for the number and often for the approximate position for side chains, but for an unequivocal solution it has been necessary to resort to chemical breakdown of the molecule and identification of the products. This has been performed (i) by the stepwise degradation of the chain from the carboxyl by standard chain shortening methods, or (ii) by cleavage of the chain at the tertiary carbon(s) by strong oxidizing agents. Method (i) may involve many operations resulting in a small yield of identifiable product, while the identification of the cleavage products from (ii) may be complicated by the occurrence of secondary oxidation products. Recently, Cason and Fessenden (1958) have briefly reported the use of gas chromatography for the identification of the primary oxidation products of branched-chain acids arising from cleavage with chromic acid in acetic acid.

Asselineau, Ryhage, and Stenhagen (1957) have employed mass spectrometric examination of the methyl esters to locate methyl branches in fatty acids, and have shown mycocerosic acid to be 2,4,6-trimethylnonacosanoic acid. This technique requires the material to be in a relatively pure state and can give no evidence for the stereochemical configuration.

* Organic Chemistry Section, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

The method described below originated from the author's observation that the carbon chains of fatty acids, especially branched-chain acids, are extensively degraded by oxidative attack with potassium permanganate in boiling acetone. With branched-chain acids this treatment progressively produces an homologous series of branched-chain acids of diminishing carbon number until the degradation reaches a methyl side chain when a methyl ketone is produced, the methyl group being that of the side chain.

Using tuberculostearic acid (10-methyloctadecanoic acid) as a simple example of a branched acid of known structure (Spielman 1934), oxidation by this method is found to produce an homologous series of branched-chain acids C_{18}–C_{11}, a C_{10}-ketone (2-decanone), and then the straight-chain acids decreasing in carbon number from C_9.

$$\begin{array}{l} CH_3(CH_2)_7CH(CH_2)_8COOH \\ \qquad\qquad\quad | \\ \qquad\qquad\quad CH_3 \\ \qquad\qquad\quad \downarrow \\ CH_3(CH_2)_7CH(CH_2)_nCOOH \qquad (n=7\rightarrow 0) \\ \qquad\qquad\quad | \\ \qquad\qquad\quad CH_3 \\ \qquad\qquad\quad \downarrow \\ CH_3(CH_2)_7C{=}O \\ \qquad\qquad\quad | \\ \qquad\qquad\quad CH_3 \\ \qquad\qquad\quad \downarrow \\ CH_3(CH_2)_mCOOH \qquad\qquad (m=7,\ 6,\ 5,\ \text{etc.}) \end{array}$$

The single ketone product and the acid mixture are readily separable, and are examined by gas chromatography, the acids as their methyl esters. The position of the branch is then firmly established by the identity of the ketone, by the absence of an acid of the carbon number of the ketone, and by the clear change in character of the acids with decrease in carbon number from branched to straight chain.

In the present paper the scope of the method is illustrated by its application to tuberculostearic acid and C_{27}-phthianoic acid (hydrogenated C_{27}-phthienoic acid).* It is also shown applied to a branched acid of lower molecular weight (3,7-dimethyloctanoic) and to the *cyclo*propane fatty acid, dihydrosterculic (9,10-methyleneoctadecanoic).

II. Experimental

(a) *General Method of Oxidation and Separation of Products*

The oxidation conditions first used, as with tuberculostearic and C_{27}-phthianoic acids below, were as follows: A solution of the acid (about 1%) in dry acetone is refluxed on a hot plate and stirred magnetically while powdered potassium permanganate is added in small amounts at intervals. Oxidation was slow and was allowed to continue for several days. It has been since found that the same result is achieved by adding the permanganate in one lot (to make a 2–4%

* These acids were isolated from the acetone-soluble fraction of tubercle bacillus wax (Kranz and Murray, unpublished data). Since several 2-methyl-2-enoic acids of different carbon number are present, confirming the findings of Cason and Fonken (1956), the nomenclature of these authors has been adopted.

solution) and refluxing overnight. When all the permanganate has been used the reaction mixture is cooled, the manganese dioxide filtered off and well washed with dry acetone or ether. The filtrate contains the neutral material (ketones) while the acids, presumably as their potassium soaps, accompany the manganese dioxide. The convenience of this separation has been previously noted by Morgan and Polgar (1957). However, for subsequent gas chromatographic examination it is desirable when possible to eliminate traces of acids in the ketone fraction and vice versa. The residue of neutral material from the filtrate is taken up in light petroleum (b.p. 40–60 °C) and chromatographed on alumina (Brockmann activity II/III) or silica gel. On alumina of this activity the ketones are not retained and are washed through by more light petroleum. On the silica gel used (British Drug Houses, London) they were eluted by a 10 : 1 light petroleum/ethyl ether mixture. Besides traces of acids this treatment separates diacetone alcohol, which is formed in small amounts from acetone under the above conditions, and is retained on the column.

The acid mixture is recovered from the manganese dioxide by dissolving the latter in sodium bisulphite and dilute hydrochloric acid, extracting the acids twice with ether, washing the ether solution twice with small amounts of water, drying over anhydrous sodium sulphate, and evaporating the solvent. The acidic residue is dissolved in aqueous alcoholic (40% alcohol) potassium hydroxide (0·5N) and the solution shaken with light petroleum to extract neutral material. When, on standing, both phases have completely cleared, the aqueous alcoholic layer is drawn off. (A hypodermic syringe has been found convenient for this purpose when working with small volumes.) The acids are liberated with dilute hydrochloric acid, extracted by ether, the solution dried over anhydrous sodium sulphate, and the solvent evaporated. The acids are methylated preferably with diazomethane in ether. Finally, to eliminate the possibility of traces of polar material in the ester mixture, it is dissolved in light petroleum and the solution passed through a short column of neutral alumina of medium activity (II/III).

(b) Gas Chromatographic Technique

(i) *Apparatus.*—The columns were heated in a temperature-controlled, well-stirred air-bath. The detector, heated independently of the columns, was a new design of the Martin and James gas density meter (Murray 1959). The sample was introduced by metal rod probes provided with calibrated capillary tips which were inserted through a valve into a preheater at 10–20 °C above the column temperature.

The column used mostly in this work was of stainless steel tubing, 8 ft in length and 0·17 in. bore, and was coiled after packing. The stationary phase (25% by wt. of the packing) was "Silicone Elastomer E301" (Griffin and George, London) and was supported on "Celite 545" graded to 60–85 mesh (B.S.S.) and water washed. This phase had only a slight volatility at the highest temperature used.

(ii) *Programmed Heating of Columns.*—When examination is being made of mixtures of esters with a wide range of carbon number, and when the lower or intermediate members are of interest, it has been found that much clearer separations are obtained by increasing the column temperature during the run while keeping the temperature of the detector constant at or just above the maximum reached. The means for doing this has been briefly described (Desty 1958). Since the reproducibility of conditions is then found to be not as good as operation at constant temperature, the reference sample of *n*-fatty acid esters is run combined with the ester sample being investigated (see Fig. 1 (*b*)).

(c) Details of Oxidations and Examination of Oxidation Products by Gas Chromatography

(Where possible the conditions used in the gas chromatographic examination are included in the explanatory text accompanying the diagrams.)

(i) *Tuberculostearic Acid.*—The sample used was of high purity (m.p. 12·5–13·0 °C, optical rotation not measurable) : a gas chromatogram of its methyl ester revealed only a trace of methyl stearate.

The acid (0·47 g) was oxidized with potassium permanganate (5 g) over a period of 36 hr and gave a ketone fraction (0·005 g) and an acid fraction (0·326 g).

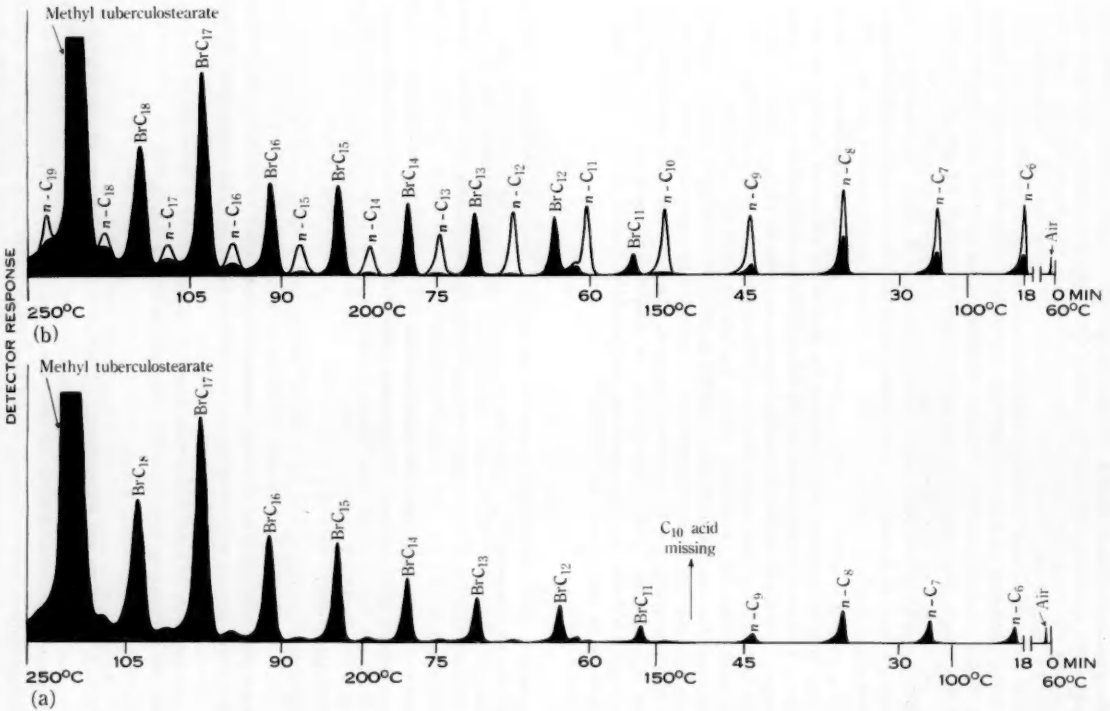


Fig. 1.—(*a*) Chromatogram of the derived esters from the oxidation of tuberculostearic acid. (*b*) The same esters with the addition of a reference series of esters of the *n*-acids C_6–C_{19}. BrC_n, branched C_n acids.
Conditions : Programmed heating of columns from 60–260 °C ; preheater temperature 265 °C ; nitrogen pressure 800 mm of mercury ; charge, 20 μl.

When examined on three stationary phases of widely different character (" Silicone E301 ", sodium dodecylbenzenesulphonate, and benzyl diphenyl), the ketone fraction was found to consist of a single component, the retention times of which agreed to within 1% of those of a synthetic specimen of 2-decanone (b.p. 208–212 °C). The identification of the ketone as 2-decanone was supported by the identity of its infra-red spectrum with that of the authentic specimen.

Figure 1 (*a*) is a chromatogram of the mixture of esters and Figure 1 (*b*) that of the same mixture combined with a series of esters of *n*-methyl acids (C_6–C_{19}).

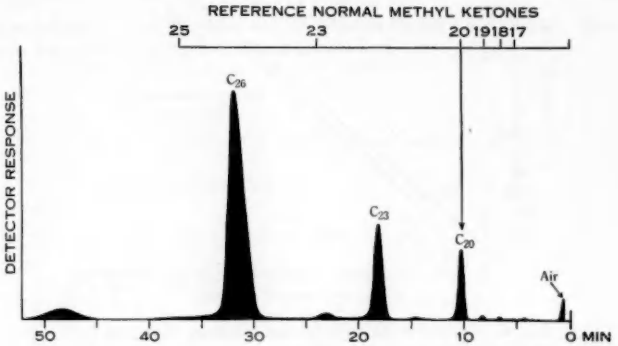


Fig. 2.—Chromatogram of the ketone fraction from the oxidation of C_{27}-phthianoic acid. The positions of members of a reference series of *n*-alkyl methyl ketones run separately are marked.

Conditions: Column temperature 265 °C; nitrogen pressure 800 mm; charge 1 μl.

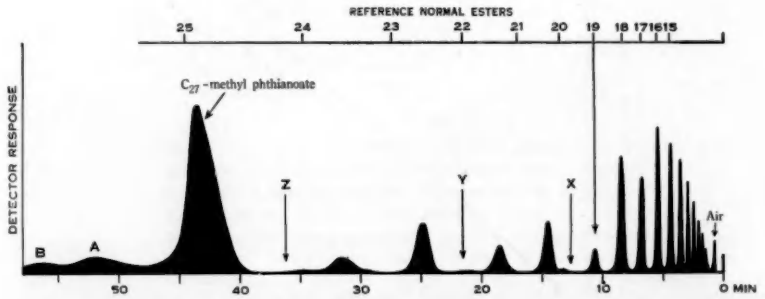


Fig. 3.—Chromatogram of the derived esters from the oxidation of C_{27}-phthianoic acid. The positions of the esters of a series of *n*-acids (oxidized *n*-hexacosanoic acid) run separately are marked. Conditions as for Figure 2.

(ii) *C_{27}-Phthianoic Acid.*—The acid used ($[\alpha]_D^{20}$, +2·7°, ultraviolet absorption at 216 mμ, ε 1200) was obtained from a sample of C_{27}-phthienoic acid (m.p. 40·6–41·5 °C, $[\alpha]_D^{20}$, +18·5°, ultraviolet spectrum $\lambda_{max.}$ at 216 mμ, ε 13050) by hydrogenation of its methyl ester. Oxidation with potassium permanganate (0·8 g) of this acid (0·080 g) over a period of 30 hr gave a ketone fraction (0·029 g) and an acid fraction (0·036 g).

A chromatogram of the ketone fraction is shown in Figure 2. The retention times of the three main peaks are compared graphically in Figure 4 with those of a series of 2-alkanones

(C_{17}, C_{20}, C_{23}, C_{25}, C_{27}) and a number of monomethyl (branched) alkyl methyl ketones. The latter were 11-methyl-2-nonadecanone, 19-methyl-2-eicosanone, 19-methyl-2-heneicosanone, 21-methyl-2-docosanone, and 25-methyl-2-hexacosanone.

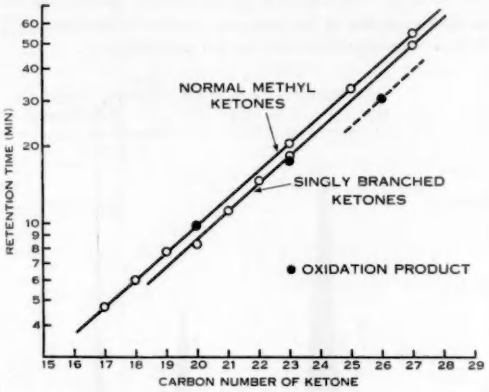


Fig. 4.—Retention times of the ketones from the oxidation of C_{27}-phthianoic acid plotted on a log scale against their carbon number, and compared with a series of *n*-alkyl methyl ketones and a number of singly-branched methyl ketones.

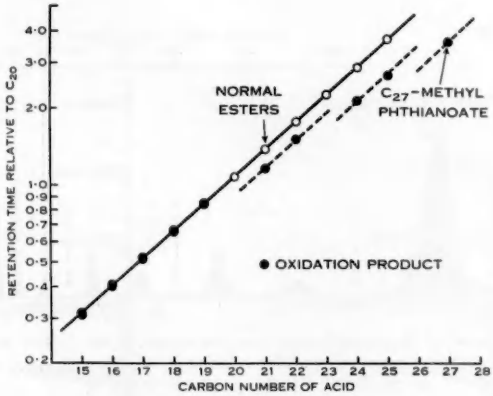


Fig. 5.—Retention times of the derived methyl esters from the oxidation of C_{27}-phthianoic acid plotted on a log scale *against* their carbon number, and compared with a series of *n*-fatty acid methyl esters.

A chromatogram of the ester fraction is shown in Figure 3 and the retention times of the peaks compared graphically in Figure 5 with those of the esters of a series of *n*-fatty acids C_{16}–C_{28}.

(iii) *3,7-Dimethyloctanoic Acid.*—This was prepared by the hydrogenation of a pure specimen of geranic acid obtained by the oxidation of citral. The acid (0·7 g) was oxidized with potassium permanganate (5 g) over a period of 24 hr giving a ketone fraction (0·054 g) and an acid fraction which was not weighed. To avoid loss of lower acids the acid mixture was not treated as described above to remove traces of ketones, and after methylation the solvent was not completely removed to avoid loss of lower esters.

The gas chromatogram showed the ketone fraction to be a single compound. It emerged between 2-heptanone and 2-octanone in the position expected for a monomethyl branched 2-heptanone.

The retention times for the esters are compared in Figure 6 with those of homologous series of esters of the *n*-acids C_5–C_{10} and of the *iso*-acids C_5–C_7.

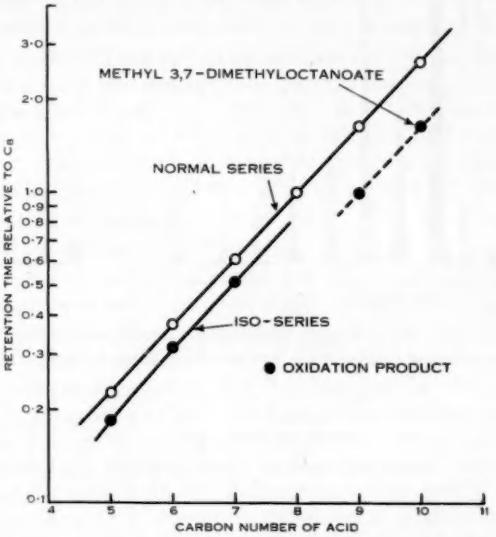


Fig. 6.—Retention times of the derived esters from the oxidation of 3,7-dimethyloctanoic acid plotted on a log scale *against* their carbon numbers. Compared with a series of esters of *n*-acids C_5–C_{10} and *iso*-acids C_5–C_7.
Conditions: Column temperature 150 °C; nitrogen pressure 300 mm; charge 2·5 μl.

(iv) *Dihydrosterculic Acid.*—The specimen melted at 38–39 °C. Gas chromatographic examination of its methyl ester showed the presence of impurities indicated by position as the C_{20} and C_{18} homologues of dihydrosterculic acid and the C_{19} and C_{18} straight-chain acids.

The acid (0·196 g) was oxidized over a period of 36 hr with potassium permanganate (4 g) giving only a trace of neutral material and an acid fraction (0·158 g).

The trace of neutral material was retained by the column when examined under conditions which would have shown the presence of methyl ketones up to C_{14}. A chromatogram of the derived esters is shown in Figure 7 in which the relative positions of the reference *n*-esters are indicated by arrows.

(v) *2-Eicosanone.*—A pure synthetic sample, m.p. 59·3–59·7 °C (obtained from Dr. R. J. Meakins)* was used.

* Division of Electrochemistry, C.S.I.R.O.

The ketone (0·172 g) was oxidized over a period of 40 hr, 0·8 g of potassium permanganate being used. The products were a small amount of acid (0·003 g) and unchanged ketone. A chromatogram of the derived esters is shown in Figure 8.

(vi) n-*Hexacosanoic Acid.*—Prepared by oxidation of a pure specimen of *n*-hexacosanol (m.p. 79·6–79·9 °C) with chromic acid in acetic acid.

The acid (0·130 g) and potassium permanganate (1·3 g) in acetone (50 ml) were refluxed overnight. The resulting mixture of acids was recovered and methylated according to the method described above.

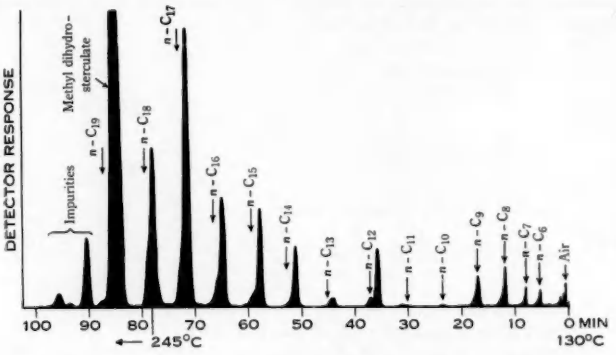


Fig. 7.—Chromatogram of the derived esters from the oxidation of dihydrosterculic acid. Conditions: Programmed heating from 130–245 °C; nitrogen pressure 500 mm; charge, 2·5 μl.

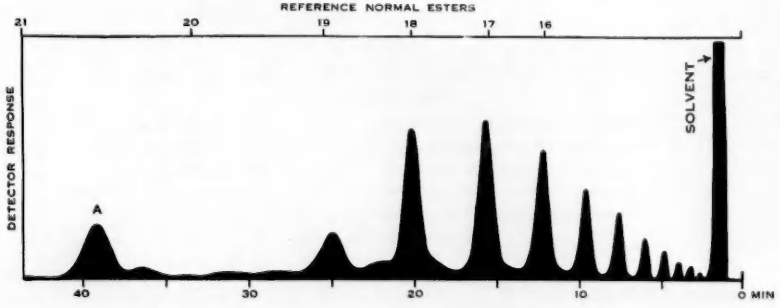


Fig. 8.—Chromatogram of the derived esters from the oxidation of 2-eicosanone, compared with the relative positions of a series of esters of the *n*-acids C_{16}–C_{21} run separately. Conditions: Column temperature 250 °C; nitrogen pressure 500 mm; charge (not known) dissolved in ether.

The derived ester mixture clearly showed the presence of homologues down to C_{15}, but the extent of degradation was very much less (approximately one-eighth) than for a sample of C_{27}-phthianoic acid oxidized under comparable conditions. This mixture of esters has been used as a reference series of *n*-fatty acid esters after checking the retention times of several members with those of the pure compounds.

III. Interpretation of Gas Chromatograms

(*a*) *Tuberculostearic Acid.*—Figures 1 (*a*) and 1 (*b*) give striking evidence that the methyl branch is attached to carbon 10. Degradation has produced the C_{18}–C_{11} homologues of this branched acid, and in the chromatogram their esters emerge alternately with the *n*-esters and between that of the same and one less carbon atom. The C_{10} acid is missing, but then the esters of a second series of acids appear, and decreasing from C_9 agree exactly with those of the normal series.

The identification of the single ketone product as 2-decanone is complementary evidence to the above. The fact that only one acid (C_{10}) is missing from the acid series is in itself evidence that there is a methyl side chain.

Additional evidence from the chromatogram for the position of the branch is that the peak area of the esters of the C_9 acid is less than that of the C_8 acid, the ratio being approximately 1 : 3. This ratio is always observed for the ester peaks of the acids with one and two carbons less than the carbon number of a ketone product. It also holds for the relative amounts of the C_{19} and C_{18} straight-chain acids obtained from the oxidation of 2-eicosanone (Fig. 8).

It is of interest to note that no traces of C_9 or C_{11} ketones were observed, so that there is no distribution of branching about the 10 position.

(*b*) *C_{27}-Phthianoic Acid.*—This material is 2,4,6-trimethyltetracosanoic acid* with small impurities of other branched acids. However, for the interpretation of the gas chromatograms it is sufficient to suppose that we are dealing with a saturated multibranched acid probably of 27 carbon atoms.

The feature most apparent in the chromatogram of the mixture of acids (Fig. 3) is that the acids produced by degradation include a series of *n*-acids decreasing from C_{19}. Between this acid and the parent C_{27} acid only four acids are represented and the inference must be that oxidation at three stages passes through a methyl ketone with the destruction of the methyl group. In fact three ketones are evident in Figure 2 as three main peaks: the smaller peaks are attributed to ketones derived from the impurities. It follows that in the degradation the first ketone produced must have two branches, the second one branch, and the third should be a *n*-alkyl methyl ketone. Likewise the interpolating acids must be doubly and singly branched.

* The C_{27}-phthienoic acid from which this acid was derived has also been investigated by the present method. The result together with other evidence which will be given in a subsequent paper supports the structure of 2,4,6-trimethyltetracos-2-enoic acid given by Polgar and Robinson (1951) to mycolipenic acid I. However, there is also evidence for the presence of one or more closely associated acids with branches more remote from the carboxyl. These impurities may explain the evidence of Cason, Urscheler, and Allen (1957) which led them to propose a structure for C_{27}-phthienoic acid with the third branch (on carbon 6) further along the chain.

Note added in Proof.—From an investigation involving the synthesis of methyl *erythro*-2,4,6-trimethyl-*trans*-$\Delta^{2:3}$-tetracosenoate and the comparison of this by mass spectrometric methods with samples of methyl mycolipenate and methyl C_{27}-phthienoate, the Swedish group of workers (Ahlquist *et al.* 1959) have concluded that " the main α,β-unsaturated acid present in ' phthioic acid ' has the 2,4,6-trimethyl-substituted structure proposed by Polgar and Robinson for mycolipenic acid I but that, in agreement with previous findings by Cason *et al.* (1956), several other components are present ".

It remains to determine where the ketones lie in the degradation sequence. The third ketone is placed as 2-eicosanone, and corresponds in carbon number to a missing acid which leaves a gap *X* in the series of ester peaks. The two other breaks in the acid series are located at *Y* and *Z*, on evidence from the relative size of the adjacent peaks (see above) and from a comparison of the retention times of the four unplaced ester peaks with those of the *n*-ester series.

The oxidation products then have the following carbon numbers:

Acids	..	27		25	24		22	21		19	18	17	16	15	etc.
Ketones			26			23			20						

The original acid therefore has methyl branches in the 2-, 4-, and 6-positions.

The validity of the above conclusion is clearer when the result is expressed (Figs. 4 and 5) in the graphical form of James and Martin (1952, 1956). These authors showed that homologous series of normal and singly-branched (*iso*- and *anteiso*-) esters, when plotted in this way, form parallel straight lines. They also observed that the branched esters have retention times between the normal ester of the same and one less carbon mumber. This also holds where the branch is elsewhere in the chain, as with the esters of tuberculostearic acid and its homologues (Fig. 1). A similar relation between singly-branched and straight-chain methyl ketones is apparent from Figure 4. This evidence leads to the conclusion that the C_{23} ketone and the C_{21} and C_{22} acids are singly branched.

Values for the retention times of doubly-branched compounds are generally lacking, but from the one example of a doubly-branched methyl ester (of 3,7-dimethyloctanoic acid, Fig. 6), the data of Desty and Whyman (1956) for lower hydrocarbons, and from boiling point evidence,* it is concluded that the plotted positions of the C_{26} ketone and the C_{24} and C_{25} acids are in agreement with their having two methyl branches.

Although it was originally assumed that the original acid had 27 carbon atoms it is probable that the result could have been derived without this knowledge as there appears to be no alternative way of interpreting the gas chromatographic evidence.

(*c*) *3,7-Dimethyloctanoic Acid.*—This acid was investigated primarily to determine whether a branched-chain acid of relatively low molecular weight could be degraded by this method. It has been found that it may, but from the relative amounts of the products it is more resistant to attack than C_{27}-phthianoic acid.

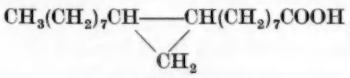
* It is well established that, on non-polar stationary phases (e.g. hydrocarbon or silicone), compounds of low polarity emerge strictly in order of their boiling points. It follows that the relation between the boiling points of branched- and straight-chain compounds will also be apparent in their relative retention times.

From a literature survey of accurate boiling points of branched- and straight-chain compounds (hydrocarbons and methyl esters) of the same carbon number it is observed that: (i) a single methyl branch lowers the boiling point so that the branched compound boils approximately midway between the straight-chain compounds of the same and one less carbon number. The decrease varies slightly according to the position of the branch, being least when in the 3-position (i.e. *anteiso*-) and most with the branch at the mid-point of the chain. (ii) Two methyl branches generally have double the effect of one, but this varies more with the positions of the branches.

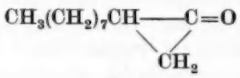
The location of the branch on carbon 3 is in keeping with the isolation of a single ketone which had a retention time for a C_8 singly-branched methyl ketone and with the results from the ester mixture plotted in the manner above (Fig. 6). The retention times of the esters of the C_7, C_6, and C_5 acids agree with those of the *iso*-acids: an expected C_4 peak for *iso*butyric acid was not obvious but possibly it was small and shielded by the trailing of the large solvent peak.

(*d*) *Dihydrosterculic Acid.*—It appears from the chromatogram of the derived mixture of esters (Fig. 7) that degradation has produced a series of acids homologous with dihydrosterculic. Their methyl esters emerge just before the *n*-ester of the same carbon number. Only traces of C_{11} and C_{10} acids are indicated. The subsequent series of acids is normal, decreasing from C_9.

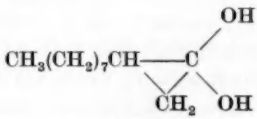
Such evidence accords with the known structure of dihydrosterculic acid (I) (Nunn 1952) which should give rise to the C_{12} to C_{18} *cyclo*propane acids and the normal acids up to C_9.



(I)



(II)



(III)

The C_{11} product should be the ketone II and by analogy with *cyclo*propanone, would be present as the monohydrate III (Brown, Fletcher, and Johannesen 1951). This would explain why no neutral fraction was obtained.

A surprising feature of the chromatogram is the small amount of the C_{13} acid. It is possible that this acid is more readily oxidized by having the methylene adjacent to the ring activated both by the ring and by the carboxyl.

Nunn (1952) found that the parent acid was not attacked by potassium permanganate in acetone, and the orderly appearance of the acids in this degradation suggests that the *cyclo*propane ring is disrupted only when by degradation the carboxyl appears on the carbon atom β to the ring.

(*e*) *2-Eicosanone.*—As noted earlier this ketone is oxidized to nonadecanoic and stearic acids but preferentially to the latter. At the same time other acids are formed the esters of which have longer retention times than the esters of the above acids. The peak of one such ester is shown in Figure 8 emerging between the esters of the C_{20} and C_{21} *n*-acids introduced as references. Another smaller peak (not shown) is situated between the positions for the esters of the C_{21} and C_{22} acids. The production of these unknown acids is not a feature of the oxidation of 2-eicosanone alone, as " foreign " peaks occur in several of the chromatograms of the ester mixtures. From their positions relative to those produced by this ketone, they are always associated with the further oxidation of a ketone product. Thus the peaks *A*, *B* in Figure 3 are due to the oxidation of the C_{26} ketone and

the shoulder on the C_{12} peak in Figure 1 (*a*) is related to the oxidation of the C_{10} ketone.

With this relation to a ketone established, no difficulty has been caused by the presence of these peaks in the interpretation of the chromatograms of the ester mixtures. The oxidation of methyl ketones is being studied to establish the structure of these unknown acidic products.

IV. Discussion

It has been known for many years that oxidation of unsaturated acids and glycerides with potassium permanganate in acetone is accompanied by some degradation of the carbon chain of the acid products, and methods have been devised to suppress consecutive oxidation. However, there appears to have been no full investigation made of the degradation of fatty acids and no attempt to use it for chain shortening.

The comparative ease with which a branched acid degrades was first observed by the author when studying the products from oxidation of C_{27}-methyl phthienoate (cf. Polgar 1954). A seemingly pure ester gave an obvious mixture of acids, and when their methyl esters were investigated by amplified distillation and later by gas chromatography, there were found to be present considerable amounts of lower acids extending in carbon number down to C_9. This led to the study of the oxidation of a number of saturated acids, both with branched and straight chains, the results from some of which are reported in this work.

Under the oxidation conditions used there appears to have been no direct attack of the branched acids at their tertiary carbon atoms. The acid product has in all cases been totally soluble in light petroleum, and it is concluded that both hydroxy acids from possible hydroxylation of the tertiary carbon(s) and dibasic acids from cleavage of the chain are not formed. Furthermore an oxidation of pure squalane (hexamethyltetracosane) by this procedure gave no traces of C_8 and C_{13} ketones which would have been expected had cleavage of the chain occurred.

It was the desire to avoid direct attack at the tertiary carbons which influenced the original choice of mild oxidation conditions, given by a dilute solution in acetone and the addition of permanganate in small amounts. It was later observed that the addition of the permanganate in one lot, and refluxing for a shorter time caused no apparent change in the products. A large proportion of the permanganate is used in oxidizing acetone, possibly by the oxidation of diacetone alcohol and mesityl oxide arising from the acetone. The relative amount of permanganate employed (10 times the weight of acid) was adequate for extensive degradation of C_{27}-phthianoic acid but probably insufficient for the lower molecular weight acids, where, as in tuberculostearic acid, the yield of ketone was small. To carry out extensive degradation on acids relatively difficult to oxidize (e.g. *n*-fatty acids), and needing large amounts of permanganate it might be necessary, owing to the bulk of accumulated manganese dioxide, to recover periodically the products from the reaction mixture and proceed with fresh solvent. As acetone is known to oxidize more readily when wet, it

would be an advantage to remove the water formed as an azeotrope with an appropriate liquid, e.g. *n*-pentane.

This method of structure elucidation has a number of advantages which merit notice:

(i) A most important feature of this method of degradation is that it provides a possible means of determining the optical configuration of all the centres of asymmetry and obviates the need to prepare optically active reference compounds through synthesis and resolution. By oxidizing somewhat larger amounts of branched acid than used in the examples above, it should be possible to isolate by amplified distillation or gas chromatography, selected compounds, the optical rotation of which could provide this information. In the case of C_{27}-phthianoic acid where the branched groups are close, it has been found possible to use the rotation of the two branched ketones, C_{26} and C_{23}, as evidence for the configuration of the asymmetric centres in the 4- and 6-positions (Murray, unpublished data).

(ii) It has been shown particularly through the oxidation of C_{27}-phthianoic acid that clear evidence of structure can be obtained from gas chromatographic examination of the products alone. It should therefore be possible to obtain valuable structural knowledge by the oxidation of only a few milligrams of unknown acid. This amount of material could easily be isolated in the first place by gas chromatography.

(iii) On the other hand the method is not entirely dependent on gas chromatography. For example, where an unknown acid has a single branch, the identification of the single *n*-alkyl methyl ketone product by other means would provide adequate proof of structure.

(iv) The acid whose structure is to be determined need not be of the highest purity. As the whole of the oxidized sample is examined by gas chromatography, impurities are usually apparent, and since the structure is deduced from the dovetailing of data from acids and ketones, their presence need not interfere with the interpretation of the results. In reverse, the method could provide a useful check on the purity of synthetic branched-chain acids.

(v) The method has an obvious application to the structure of *cyclo*propane fatty acids and it is possible that carbon chains attached to other ring structures may be likewise degraded.

(vi) The oxidation of long-chain acids (e.g. *n-iso-* or *anteiso-*) on a larger scale and the isolation of the esters by preparative gas chromatography is a convenient method for the preparation of homologous series of acids of high purity for reference purposes.

(vii) A possible extension of this method is in the study of biosynthesis of branched- or straight-chain fatty acids. The positions of radioactive carbon incorporated into the chain could be located by degrading the saturated acids and correlating the amount of each ester with a β-ray count.

(viii) Except for 3,7-dimethyloctanoic acid the method has not been applied to acids with a terminal *iso*propyl group. With such structures no information

can be obtained from the expected ketone product (acetone), but the branched position should be apparent from the ester chromatograms. It is possible that an alternative solvent could be used to allow the detection of the produced acetone.

V. Acknowledgment

The assistance of Mr. Z. H. Kranz is gratefully acknowledged.

VI. References

Ahlquist, L., Asselineau, C., Asselineau, J., Ställberg-Stenhagen, S., and Stenhagen, E. (1959).—*Ark. Kemi* **13**: 543.

Asselineau, J., Ryhage, R., and Stenhagen, E. (1957).—*Acta Chim. Scand.* **11**: 196.

Brown, H. C., Fletcher, R. S., and Johannesen, R. B. (1951).—*J. Amer. Chem. Soc.* **73**: 212.

Cason, J., Allen, C. F., Deacetis, W., and Fonken, G. J. (1956).—*J. Biol. Chem.* **220**: 893.

Cason, J., and Fessenden, J. (1958).—Abstr. Pap. presented at meeting of Na[illegible]al Academy of Science, Berkeley, Cal., November 1958. *Science* **128**: 1142.

Cason, J., and Fonken, G. J. (1956).—*J. Biol. Chem.* **220**: 391.

Cason, J., Urscheler, H. R., and Allen, C. F. (1957).—*J. Org. Chem.* **22**: 1284.

Desty, D. H. (1958).—"Gas Chromatography 1958." (Ed. D. H. Desty.) p. 246. (Butterworths Scientific Publications: London.)

Desty, D. H., and Whyman, B. H. F. (1957).—*Analyt. Chem.* **29**: 321.

James, A. T., and Martin, A. J. P. (1952).—*Biochem. J.* **50**: 679.

James, A. T., and Martin, A. J. P. (1956).—*Biochem. J.* **63**: 144.

Morgan, E. D., and Polgar, N. (1957).—*J. Chem. Soc.* **1957**: 3780.

Murray, K. E. (1959).—*Aust. J. Appl. Sci.* **10**: 156.

Nunn, J. R. (1952).—*J. Chem. Soc.* **1952**: 313.

Polgar, N. (1954).—*J. Chem. Soc.* **1954**: 1008.

Polgar, N., and Robinson, R. (1951).—*Chem. & Ind.* **1951**: 685.

Spielman, M. A. (1934).—*J. Biol. Chem.* **106**: 87.

THE PREPARATION OF THE ESTERS OF SOME SUBSTITUTED SEBACIC ACIDS FROM OLEIC ACID

By L. K. Dalton,* H. H. Hatt,* and A. Z. Szumer*

[*Manuscript received May* 18, 1959]

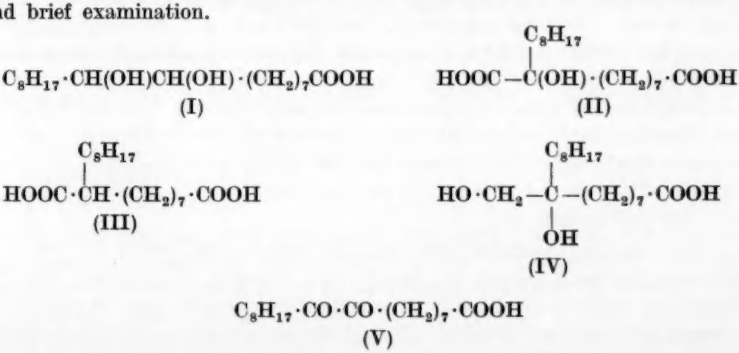
Summary

threo-9,10-Dihydroxyoctadecanoic acid (I) like its erythro-isomer gives good yields of 2-hydroxy-2-octyldecandioic acid (II) when fused with alkali. Under similar conditions 9,10-diketo-octadecanoic acid gives a somewhat lower yield of this same acid and is considered a probable intermediate in the conversion of I to II.

Esters of II have been tested as lubricants at low temperatures and as plasticizers. They are too heat sensitive to serve as lubricants. The octyl side chain renders them less effective as plasticizers than esters of sebacic acid. The 2-ethylhexyl ester of 2-octyldecandioic acid (III), derived from II, has properties which fit it for use as a lubricant.

I. Introduction

Esters of sebacic acid are valued as components of lubricants for use over an extended range of temperature and as plasticizers for polyvinyl chloride. The major source of sebacic acid is castor oil, although its preparation through the electrolysis of a half-ester of adipic acid is now technically feasible. Long ago Le Sueur (1901) made a remarkable observation which opens a route from oleic acid to the hydroxyoctylsebacic acid (II). He found that *erythro*-9,10-dihydroxyoctadecanoic acid (I) rearranged in fused alkali to produce a hydroxy dibasic acid, which he and Withers later (Le Sueur and Withers 1914) showed to be the substituted sebacic acid (II), that is 2-hydroxy-2-octyldecandioic acid. When heated II dehydrates to give principally a mixture of olefinic dibasic acids from which 2-octyldecandioic acid (III) is readily obtained by hydrogenation. It seemed to us that esters of acids II and III could have properties suiting them for use as lubricants and plasticizers. This account deals with their preparation and brief examination.



(I) (II) (III) (IV) (V)

* Organic Chemistry Section, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

Le Sueur and Withers prepared the *erythro*-9,10-dihydroxyoctadecanoic acid (I) by oxidation of oleic acid with permanganate. Today it is more conveniently prepared by oxidation of the isomeric elaidic acid with a per acid. Its threo isomer is the more accessible, being readily prepared by oxidation of oleic acid with performic acid and this oxidation is now a commercial process. We have found that the threo-isomer produces equally good yields of II in fused alkali, and, because of its lower melting point, is more easily manipulated. Le Sueur and Withers rearranged the erythro-isomer (I) by heating gradually with 70 per cent. aqueous potassium hydroxide to 250 °C and maintaining the melt at that temperature for up to an hour. Better and much more consistent yields result if the molten acid is added to the vigorously stirred molten alkali, and the reaction mixture quenched after a few minutes. Frothing, which Le Sueur found troublesome, is diminished if the aqueous alkali is replaced by a 35 : 65 mixture of potassium and sodium hydroxides. An excess of alkali is needed to serve as the reaction medium, though most of it may be later recovered by the addition of a little water, when the reaction mixture separates into two phases, one containing the salts of the organic acids and the other the unused alkali. This latter aqueous layer is practically free of organic material.

By this procedure a 60 per cent. yield of pure II is readily obtained. Chitwood (1945) claimed that cadmium oxide added to the fused alkali accelerated the dehydrogenation of primary alcohols to carboxylic acids and improved the yield. Its use here also improved the yield and purity of II, as well as increasing the rate of rearrangement. With its aid yields of 70 per cent. have been obtained. There is reason to believe that at higher temperatures and with decreased reaction times such as are possible in a continuous process, still better yields would arise.

II. Esters of 2-Hydroxy-2-octyldecandioic Acid (II)

The esters were made by refluxing the acid with the appropriate alcohol and sulphuric acid. The properties of certain of these esters as plasticizers of polyvinyl chloride are given in Table 1. Evaluation was in accordance with British Standard Specification 2571/1955. The plastic mixture used had the following composition by weight: PVC, 67;* ester as plasticizer, 33; basic lead carbonate, 1; calcium stearate, 1. Compared with di-2-ethylhexyl sebacate itself they are inferior in cold flex properties, but somewhat better than esters of phthalic acid. They were also less compatible as shown by the longer milling times needed and by some exudation observed after storage. The diethyl ester, which approximates in molecular weight to di-2-ethylhexyl sebacate was the most easily compounded, and showed the best overall performance of the esters examined. The esters of II improved the heat stability of the PVC but detailed testing was not undertaken.

For evaluation as engine lubricants the esters were tested according to specification D.Eng.R.D.2487 of the British Ministry of Supply. The di-*iso*butyl ester failed to meet the requirements before heating and the di-2-ethylhexyl ester just met them (see Table 2). However, esters of II dehydrate readily

* I.C.I.A.N.Z. "Corvic D65/2".

when heated at 220 °C and hence fail in their properties as lubricants after heating at 280 °C. After heating, the di-2-ethylhexyl ester has the properties of a gel at −40 °C.

TABLE 1

THE PLASTICIZING PROPERTIES* OF ESTERS OF 2-HYDROXY-2-OCTYLDECANDIOIC ACID (II)

Ester	Cold Flex Temperature (°C)	B.S. Softness Number	Tensile Strength (lb/in²)	Elongation at Failure (%)
Diethyl	−19	23	2760	330
Di-*n*-butyl	−23	14	2630	420
Di-*iso*butyl	−18	17	2670	430
Di-(2-ethylhexyl) sebacate ..	−37	30	2630	340
Di-(2-ethylhexyl) phthalate..	−10	30	2860	390

* Determined in accordance with B.S.S. 2571/1955.

III. ESTERS OF 2-OCTYLDECANDIOIC ACID (III)

2-Octyldecandioic acid was prepared by dehydrating 2-hydroxy-2-octyldecandioic acid, followed by hydrogenation under pressure in glacial acetic acid with platinum oxide as catalyst. Dehydration was followed by measurement of absorption at 222 mμ and appeared to be completed in 2 hr at 220 °C. Esters

TABLE 2

VISCOSITIES OF ESTERS

Ester	Viscosity (cS) before Heating			Viscosity (cS) after Heating*		
	−40 °C	37·8 °C	98·9 °C	−40 °C	37·8 °C	98·9 °C
Di-(2-ethylhexyl) 2-octyldecandioate	4120	23·0	4·8	3960	23·0	4·8
Di-(2-ethylhexyl) 2-hydroxy-2-octyldecandioate	11820	28·7	5·4	Gels	—	—
Di-*iso*butyl 2-hydroxy-2-octyldecandioate	20060	33·8	5·9	—	—	—
Di-(2-ethylhexyl) sebacate†	1410	12·8	3·3	1410	12·7	—
Specification D.Eng.R.D.2487‡	> 13000	7·5 to 39		Change	> −10 to +20%	

* Heated 24 hr at 280 °C under nitrogen.

† Technical grade.

‡ British Ministry of Supply.

of the acid thus prepared gave satisfactory analytical data, but contained a small amount of impurity which charred at 280 °C. The impurity was ketonic in nature and removable either by treatment with Girard's Reagent T or by distillation under reduced pressure. The properties of the di-2-ethylhexyl ester are listed in Table 2 and are compared there with those of di-2-ethylhexyl sebacate. They fit it for use as a lubricant.

IV. Mechanism of the Rearrangement

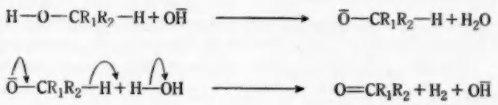
Two courses for the conversion of I to II have been proposed. Le Sueur and Withers (1914, 1915) and Le Sueur and Wood (1921) believed there was first rearrangement to the primary tertiary glycol (IV) followed by dehydrogenation. The reasons for this view appear to have been that, first, primary alcohols are known to be readily converted to acids under the reaction conditions used (Dumas and Stas 1840; see also Grundmann 1948), and, second, that they observed but little evolution of hydrogen during the first stages of the reaction, when they supposed the intermediate IV was being produced. They believed the hydrogen was evolved later and arose from consecutive reactions of II. Nicolet and Jurist (1922) on the other hand considered the initial stage of the reaction to be a dehydrogenation to the diketo acid (V) which then underwent a benzil-benzilic acid type of rearrangement.

The few preliminary experiments we have made support the view of Nicolet and Jurist. They are described here, but in view of the announcement by Weedon (1958) that he is now investigating the reaction mechanism in detail, no further work of this kind is being done. Contrary to Le Sueur and Withers, under the conditions of reaction we used, as soon as the reagents were mixed there was vigorous evolution of hydrogen which quickly diminished. Their statement that poor yields of II resulted if considerable hydrogen was evolved could not be substantiated. Early appearance of hydrogen is consistent with the intermediate formation of V rather than of IV. It is also difficult to devise a reaction mechanism for the production of IV.

In support of their mechanism, Nicolet and Jurist reported that the diketo acid (V) gave a small yield (12 per cent.) of the hydroxydibasic acid (II) when fused with alkali at 160 °C. Besides the small yield, a further weakness in their evidence was the failure to convert V to II at any higher temperatures (190 and 225 °C), although they were able to convert I to II only above 200 °C. By contrast we have found that conversion of V to II occurs readily at higher temperatures, and at 230 °C, under conditions where I yields 70 per cent. of II, compound V gives a 40 per cent. yield. Nicolet and Jurist also reported some reduction of V to the *erythro*-form of I when fused in alkali. This we have not confirmed.

The desired product II is not a stable material under the reaction conditions and when left in fused alkali at 230 °C for 1 hr over 50 per cent. was decomposed. This shows the desirability of the method of preparation we have used. After the hour, 7 per cent. was recovered as 9-ketoheptadecanoic acid, but the major product was a yellow oil with an intensity of absorption at 220 mμ equivalent to the formation of some 20 per cent. of olefinic C_{18} dibasic acids. Like Le Sueur and Withers we found azelaic and nonoic acids were constant products of the reaction in fused alkali. These could arise from competitive fission of 9,10-diketo-octadecanoic acid (V) with subsequent oxidation, analogous to the fissions of benzil and of diketosuccinic acid. However, other unidentified products are formed and may arise, as Nicolet and Jurist suggest, from condensations similar to those observed with diacetyl by von Pechmann.

The evidence at present is consistent with a mechanism in which the first stages comprise the conversion of the dihydroxy acid (I) to the diketo acid (V) by a twice-repeated dehydrogenation:



Thereafter the rearrangement parallels that of benzil to benzilic acid. As might be expected, at these high temperatures the threo- and erythro-isomers of I behave similarly.

V. Experimental

(a) *The Preparation of 2-Hydroxy-2-octyldecandioic Acid (II)*

A wide silver test tube was used and was heated in a vapour bath. Boiling safrole, diethylene glycol, and eugenol gave reaction temperatures of approximately 230, 240, and 250 °C respectively. The reaction mixture could be stirred vigorously with a silver stirrer. A mixture of alkali metal hydroxides (e.g. KOH : NaOH = 35 : 65) of 5–10 times the weight of the dihydroxy acid was used.

Table 3

THE YIELDS OF 2-HYDROXY-2-OCTYLDECANDIOIC ACID*

Composition of Alkali (% KOH)	Wt. Ratio Alkali to Acid I	Mole Ratio CdO to Acid I	Reaction Temperature (°C)	Reaction Time (min)	Yield of Hydroxy Dibasic Acid II† (%)	Purity of Product‡ (%)
60	10	Nil	180	90	11	25
50	10	,,	200	30	29	58
50	5	,,	200	90	37	96
40	5	,,	230	10	43	83
40	5	,,	230	20	51	95
40	5	,,	230	60	25	92
35	5	,,	240	5	44	90
30	5	,,	250	5	53	95
30	5	,,	250	8	58	98
35	10	0·10	240	5	70	97
35	5	0·10	240	5	67	97

* Experiments with 5 g of *threo*-9,10-dihydroxyoctadecanoic acid (I).

† 2-Hydroxy-2-octyldecandioic acid.

‡ Yields are corrected for purity.

The *threo*-9,10-dihydroxyoctadecanoic acid was added molten to the fused alkali in about a minute. A brisk evolution of hydrogen commenced immediately. After the chosen reaction time, usually a few minutes, stirring was interrupted and the mixture was cooled quickly to below 100 °C, a little water added, and then the two layers separated. The upper layer of soaps was dissolved in water, and made acidic with hydrochloric acid. The liberated organic acids were freed of azelaic acid and of most of the volatile lower fatty acids by boiling with water. The insoluble acids remaining were dried. They consisted principally of 2-hydroxy-2-octyldecandioic acid, but

a certain amount of unchanged dihydroxy acid (I) was always present. The proportion of I present was estimated from the equivalent weight; with good reaction conditions it could be held below 3%. If remaining in large quantity it is removed only with the greatest difficulty; when present in small quantity it can be removed by crystallization from 1 : 1 chloroform–*cyclo*hexane and this method of purification was used.

The reaction mixture is heterogeneous and the principal requirement for full use of the starting material is vigorous stirring. As indicated in discussing the mechanism of the reaction, prolonged reaction times are undesirable. The results of typical experiments are summarized in Table 3. Each result listed represents the mean of five or more runs. The best conditions are those in the last two rows of Table 3 with a reaction temperature of 240 °C and use of cadmium oxide. The use of greater excesses of alkali favours the reaction. At 250 °C the reaction is so rapid that in these batch processes the yields were difficult to reproduce. In a continuous process at these higher temperatures, with shorter reaction times still better yields may be possible. At lower temperatures the reaction was slower and apparently also slower relative to the ensuing destructive reactions. Nevertheless, contrary to the statement of Nicolet and Jurist, the rearrangement of I to II does occur below 200 °C.

The 2-hydroxy-2-octyldecandioic acid prepared in this way and crystallized as stated (10 ml solvent/g acid) was white and of m.p. 109–110 °C (Found: C, 65·3; H, 10·5%. Calc. for $C_{18}H_{34}O_5$: C, 65·4; H, 10·4%).

Some conversions of I to II were made in aqueous sodium hydroxide under pressure. It was then possible to use much less alkali, but yields were lower because of the increased production of by-products. The highest yield (37%) of an acid of 86% purity was obtained when a mixture of dihydroxyoctadecanoic acid (1 part), sodium hydroxide (1 part), water (2·5 parts), and cadmium nitrate (0·04 part) was heated in an autoclave at 250 °C for 30 min.

(*b*) *2-Octyldecandioic Acid* (*III*)

2-Hydroxy-2-octyldecandioic acid (30 g) was heated at 220 °C under reduced pressure (50 mm) for 2 hr, by which time absorption at 222 mμ had reached a maximum value. The unsaturated acids produced were hydrogenated without purification. In acetic acid over platinum oxide at room temperature and under a pressure of 1500 lb/in² hydrogenation was complete in 7 hr. The product, crystallized from hexane (60 ml) and then from 1 : 9 chloroform–hexane, melted at 70 °C. Yield, 23 g (Found: C, 68·9; H, 10·9; O, 20·6%; equiv. wt., 159. Calc. for $C_{18}H_{34}O_4$: C, 68·8; H, 10·9; O, 20·4%; equiv. wt., 157).

(*c*) *Preparation of Esters*

(i) *Di-*iso*butyl 2-Hydroxy-2-octyldecandioate.*—The acid (II; 45 g) and *iso*butanol (200 g) together with toluene (40 g) and sulphuric acid (2 g) were refluxed until 5·5 ml of water had been entrained. The reaction mixture was then washed with water to remove the acid catalyst and the excess of *iso*butanol was removed at 80 °C under reduced pressure. The ester remaining possessed an acid value of 1 (Found: C, 70·8; H, 11·4%. Calc. for $C_{26}H_{50}O_5$: C, 70·5; H, 11·4%).

(ii) *Di-2-ethylhexyl 2-Octyldecandioate.*—The method described under (i) was followed except that the acid III (45 g) was used with 2-ethylhexanol (150 ml) and 5·8 ml of water was entrained. The crude ester was distilled and the major portion, boiling between 232 and 238 °C at 0·4 mm Hg, was collected. It was a colourless oil, d_4^{25} 0·8969, n_D^{25}, 1·4510 (Found: C, 75·5; H, 12·1%. Calc. for $C_{34}H_{66}O_4$: C, 75·8; H, 12·4%). This ester underwent little change when heated for 24 hr at 280 °C. If the ester was not distilled, then during the heat stability test for evaluation as a lubricant, it darkened and charred a little. The impurity responsible for charring was ketonic in character and instead of removing by distillation could be removed by extraction with Girard's Reagent T.

VI. Acknowledgments

We are indebted to Mr. S. Mosbauer for much help with the experimental work.

VII. References

CHITWOOD, H. C. (1945).—U.S. Pat. 2,384,817 (Sept. 18). (*Chem. Abstr.* **40**: 354.)
DUMAS, J. B., and STAS, J. S. (1840).—*Liebigs Ann.* **35**: 132.
GRUNDMANN, C. (1948).—*Chem. Ber.* **81**: 511.
LE SUEUR, H. R. (1901).—*J. Chem. Soc.* **79**: 1313.
LE SUEUR, H. R., and WITHERS, J. C. (1914).—*J. Chem. Soc.* **105**: 2800.
LE SUEUR, H. R., and WITHERS, J. C. (1915).—*J. Chem. Soc.* **107**: 736.
LE SUEUR, H. R., and WOOD, C. C. (1921).—*J. Chem. Soc.* **119**: 1697.
NICOLET, B. H., and JURIST, A. E. (1922).—*J. Amer. Chem. Soc.* **44**: 1136.
WEEDON, B. C. L. (1958).—*Chem. & Ind.* **1958**: 402.

TERPENOID CHEMISTRY

II. DYSOXYLONENE AND δ-CADINENE

By R. P. HILDEBRAND* and M. D. SUTHERLAND†

[*Manuscript received May* 26, 1959]

Summary

The sesquiterpene, dysoxylonene, has been isolated and characterized as the diepoxide, m.p. 86–87 °C, and is shown to be nearly racemic δ-cadinene (cadina-4,10(1)-diene).

I. INTRODUCTION

The sesquiterpene, dysoxylonene, was first isolated in impure form from the wood oil of *Dysoxylon frazeranum* Benth. (rosewood) by Penfold (1927). Dysoxylonene however, unlike so many of the named sesquiterpenes described in the literature, has an individuality which can be demonstrated by the preparation of a crystalline derivative, in this case an optically inactive dihydrochloride, m.p. 108–109 °C. By dehydrohalogenation, Penfold obtained an optically inactive hydrocarbon which yielded cadalene on dehydrogenation. Despite the implication of identity between the natural and the regenerated hydrocarbons, no evidence was presented on this point.

More recently, Hellyer and McKern (1956) described the infra-red spectrum of dysoxylonene dihydrochloride (for which they found m.p. 105–106 °C) as identical with that of the well-known cadinene dihydrochloride (m.p. 119 °C, $[\alpha]_D^{20}$ −37·5). They conclude that dysoxylonene is an optically inactive cadinene.

The term cadinene is a generic one for the nine double-bond isomers, recently reviewed by Herout and Sykora (1958), which can give rise to cadinene dihydrochloride (+), (−), or (±). Thus the particular cadinene contained in *D. frazeranum* oil is not indicated or characterized by the isolation of the dihydrochloride nor by the crystalline nitrosochloride obtainable from regenerated dysoxylonene (Hellyer 1957, personal communication). Our aim was to isolate the natural hydrocarbon, to obtain derivatives characteristic of that cadinene isomer, and to determine its structure.

II. THE ISOLATION OF DYSOXYLONENE

The blue oil obtained by the steam distillation of rosewood shavings was found, in a preliminary distillation, to contain a fraction, b.p. *c.* 133 °C, which yielded dysoxylonene dihydrochloride. A wide-boiling cut containing this fraction was subjected to high efficiency distillation in a Podbielniak still at 10 mm pressure and high reflux ratio (50). A boiling point plateau was observed at 133 °C, the fractions of which gave moderate yields (*c.* 55 per cent.) of dihydro-

* Chemistry Department, University of Queensland, Brisbane; present address: Carlton and United Breweries, Melbourne.

† Chemistry Department, University of Queensland, Brisbane.

chloride (see Table 1). Consideration of the refractive index–density curve for the plateau fractions does not permit the customary claim that the sesquiterpene is " pure " since it shows several changes of direction within the plateau (neglecting irregularities due to reflux ratio variations and errors in the measurement of the physical constants). Although a rather difficult example to interpret (Sutherland 1952), it is consistent with the presence of only one major component and three minor components. The ultraviolet absorption spectrum of the bulked plateau fractions (40–60) exhibits a maximum (log ε 3·32) at 247 mμ indicative

TABLE 1

DISTILLATION FRACTIONS FROM DYSOXYLON FRAZERANUM WOOD OIL

Fraction Number	Boiling Point (°C/10 mm)	n_D^{25}	d_4^{25}	α_D^{23} (10 cm hom.)	Yield of Dihydrochloride (%)
23	129·5–130	1·5063	0·9185	+23·7°	
25		1·5064	0·9185	+23·0	
27		1·5068	0·9178	+18·4	
29	—131	1·5068	0·9177	+15·5	
31		1·5070	0·9173	+11·9	
34		1·5071	0·9168	+7·8	
36	—131	1·5072	0·9168	+4·5	(Red oil only)
37	—131·5				
38	—133	1·5087	0·9169	—0·8	
40		1·5091	0·9168	—3·1	53
42		1·5093	0·9167	—4·8	
44		1·5099	0·9165	—6·6	64
46		1·5098	0·9159	—6·0	
48		1·5099	0·9147	—7·0	
50	—133	1·5100	0·9147	—6·7	55
52		1·5102	0·9152	—7·1	
54		1·5107	0·9152	—8·5	
56		1·5106	0·9152	—9·1	
58		1·5110	0·9164	—9·1	56
60		1·5114	0·9176	—8·3°	26 (Fr. 61)
62	Sharp rise	1·5182	0·9265		

of possible 10 per cent. of a non-homoannular diene. On the other hand, gas chromatography of fraction 50, which probably differed only slightly in composition from the bulked fractions, showed one major peak (*c.* 90 per cent. of the total area) and two minor peaks. As might be expected, the gas chromatography column (4 ft of " Apiezon M " at 170 °C, Scott microflame detector) failed to resolve all of the components of the mixture of very close-boiling and chemically similar substances. The infra-red spectrum provides evidence of a trisubstituted bond and an exocyclic methylene group but the intensity of the latter band is relatively weak, indicating that it arises from a minor component. Ozonolysis by a semiquantitative technique yielded 7 per cent. of formaldehyde as the dinitrophenylhydrazone, a result which limits the component with the $=CH_2$ group to about 10 per cent. of the whole.

The purity of the bulked fractions (hereafter dysoxylonene concentrate) is thus rather indefinite, being probably about 80–90 per cent. Attempts to prepare a nitrosochloride, nitrosate, nitrosite, and tetrabromide yielded oils only. The dihydrochloride described by Penfold (1927) was readily obtained but the m.p. was found to be 105–106 °C, in agreement with Hellyer and McKern. A dihydrobromide, m.p. 125 °C, was also readily obtained from dysoxylonene concentrate. A comparison of the infra-red spectrum of dysoxylonene dihydrochloride and that of authentic (−)-cadinene dihydrochloride led us also to the conclusion that the two spectra were identical.

Neither of the above derivatives characterizes the particular isomer of cadinene which is referred to here as dysoxylonene, but eventually however, the crystallization of the diepoxide permitted both the characterization of dysoxylonene and the determination of its structure.

III. Dysoxylonene Diepoxide

Chromatography of the crude oily diepoxide obtained by the action of perphthalic acid on dysoxylonene concentrate, yielded three main bands, one of which crystallized as needles of dysoxylonene diepoxide, m.p. 86–87 °C, $[\alpha]_D \pm 0 \cdot 0°$.

A method for the determination of ethylenic bond positions (Ruzicka and Sternbach 1940 ; Campbell and Soffer 1942) which has been widely used in the sesquiterpene and diterpene series, involves the addition of methyl magnesium iodide to the derived epoxide, followed by dehydration and dehydrogenation to a characteristically methylated aromatic hydrocarbon. When applied to dysoxylonene, the method failed, apparently through lack of reactivity of the diepoxide to the Grignard reagent, since the products included unchanged epoxide and a gum containing some α-glycol, the analysis of which suggests that the introduction of possibly one methyl group had occurred. In any case, only cadalene was isolated after dehydration and dehydrogenation.

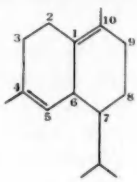
This inertness towards methyl magnesium iodide parallels the behaviour of the dextrorotatory cadinene diepoxide described by Herout and Santavy (1954) who also obtained unchanged epoxide and a rather indefinite glycol which could be dehydrogenated to cadalene.

Through the courtesy of Dr. V. Herout, who provided us with a sample of this cadinene diepoxide, we were able to make a comparison of the infra-red spectra in Nujol and hexachlorobutadiene solutions. Complete correspondence of peak positions in the two spectra demonstrated the probability that dysoxylonene diepoxide is the racemic form of the diepoxide, m.p. 83 · 5–84 · 5 °C, $[\alpha]_D^{20}$ +13 · 3°, prepared by Herout and Santavy from a citronella oil cadinene. The structure I (δ-cadinene or cadina-4,10(1)-diene) assigned to this hydrocarbon, was based largely on the failure of the Grignard reagent and methyl lithium to add to the diepoxide, the two double bonds being therefore assigned to sterically hindered positions consistent with the infra-red spectrum and the formation of cadinene dihydrochloride.

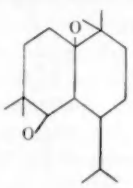
To demonstrate the structure of the diepoxide in a more convincing manner, isomerization to III was attempted with the object of then dehydrogenating III

to IV. Small samples of the diepoxide were treated with *p*-toluenesulphonic acid or sulphuric acid in ethanol or acetic acid at various temperatures. No conditions were found which resulted in appreciable amounts of III accumulating in the reaction mixture as determined by the intensity of absorption near 300 mμ.

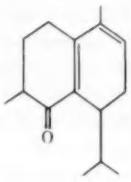
However, a peak of moderate intensity (ε 2680) slowly developed at 272 mμ and since the absorbing substance was removed from *iso*-octane solution by extraction with Claisen's alkali but not by aqueous alkali, the presence of the cryptophenol (VI) was indicated. Confirmation was provided by a positive Gibb's test and the formation of an azo dye with diazotized sulphanilic acid. However, the isomerization when carried out on a preparative scale, yielded only a mere trace of phenolic material, a crystalline substance, m.p. 186·5–187 °C,



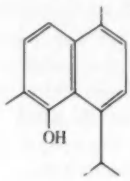
(I)



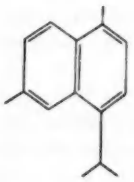
(II)



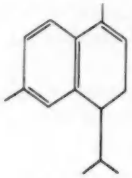
(III)



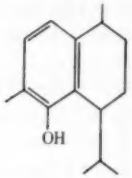
(IV)



(VII)



(V)



(VI)

which is probably an epoxydiol and an oily material, $\lambda_{max.}$ 271 mμ which is probably largely a dihydrocadalene such as V, a view supported by the ultraviolet absorption spectrum before and after hydrogenation. The apparently satisfactory yields of phenolic material indicated by the exploratory experiments, resulted from the transfer of some V as well as VI to the Claisen's alkali during the extraction.

The ketone (III) not being realizable and the phenol (IV) obtainable in unworkable yields only, a more direct and successful approach was tried in the dehydrogenation of the diepoxide itself. The literature does not provide examples of the dehydrogenation of hydroaromatic epoxides but ketones and secondary alcohols are known to yield phenols, naphthols, etc. (Linstead and Michaelis 1940; Plattner 1942), the use of an inert solvent such as *p*-cymene usually increasing the phenol to hydrocarbon ratio. It was anticipated that the dehydrogenation of II would result in the formation of the naphthol (IV) as well as the hydrocarbon cadalene (VII). A dehydrogenation of the diepoxide carried out in refluxing cymene was unsatisfactory since the presence of cymene

added to the difficulty of separating the naphthol (IV), which is reported by Plattner and Magyar (1941*a*, 1941*b*) to be insoluble even in Claisen's alkali, from the more abundant cadalene.

To avoid these difficulties and to conserve diepoxide, a microdehydrogenation technique was devised which may prove valuable in other applications also. The epoxide (10 mg) is dehydrogenated with 10 per cent. palladized charcoal in a spectroscopically clean reflux tube. The whole dehydrogenation mixture is transferred to a flask containing *iso*-octane (10 ml) and water (50 ml). Refluxing under an oil trap for 1 hr quantitatively transfers the cadalene and hydroxycadalene to the *iso*-octane layer in the oil trap. This solution is suitably diluted and its absorption spectrum examined in the ultraviolet. The optimum conditions for the dehydrogenation of dysoxylonene diepoxide to naphthol were found to be heating for 1 hr in a bath at 265 °C.

For the purposes of comparison, the ultraviolet absorption spectrum of 5-hydroxycadalene, which has not been reported in the literature and that of cadalene which is described in two grossly conflicting reports (Morton and de Gouveia 1934; Ruzicka, Schinz, and Muller 1944), were required. Cadalene from the dehydrogenation of cade oil, was regenerated from the picrate and the trinitrobenzolate by chromatography on alumina to yield identical results (Fig. 1, see Section VI) which were also in substantial agreement with Ruzicka's results as read from a small graph. 5-Hydroxycadalene prepared from guaiol by the method of Plattner and Magyar (1941*a*, 1941*b*) was crystallized as the picrate until successive crops gave identical spectra (Fig. 1 and see Section VI) for the naphthol.

Characteristic features of the absorption curves of both these substances are seen in the ultraviolet spectrum of the steam-volatile dehydrogenation products, which is plotted in Figure 1 as ε', a pseudo-extinction coefficient. This is calculated by assuming (not correctly) that the molar quantity of the steam-volatile dehydrogenation products in the *iso*-octane solution is identical with the quantity of epoxide submitted to dehydrogenation. This curve is a convenient one in that if, for example, the 338 mμ peak is assumed to be wholly due to 5-hydroxycadalene, then the percentage yield of naphthol is $100\ \varepsilon'/\varepsilon$. From the values of ε' at 338 and 296·5 mμ (an isobestic point for cadalene and 5-hydroxycadalene) the yields of naphthalene and naphthol may be calculated to be 18 and 6 per cent. respectively. However, comparison of the calculated curve for this mixture and the observed curve shows that one or more other substances are present, and it is necessary to assume that one of the unknown substances shows some absorption at the 296·5 mμ isobestic point. Reduction of the cadalene percentage by about one-tenth brings about a reasonable fit and leaves some residual absorption with a maximum in the 240–260 mμ region which is possibly due to an unrecognized conjugated ketone.

A preparative-scale dehydrogenation yielded a mixture of products separated by chromatography into cadalene (21 per cent. yield), 5-hydroxycadalene (6 per cent. yield), and a non-volatile resinous material. The epoxydiol, m.p. 187 °C, previously obtained by the hydration of the diepoxide, was also isolated (9 per cent. yield) from the products of the dehydrogenation. The 5-hydroxy-

cadalene was characterized by the ultraviolet absorption spectrum and by the picrate and the trinitrobenzolate which were identical with authentic specimens. The absence of 2-hydroxy- and 7-hydroxycadalene from the dehydrogenation products is confirmed by the observation that the ultraviolet spectrum of the crude volatile dehydrogenation products, is not altered by extraction with aqueous alkali.

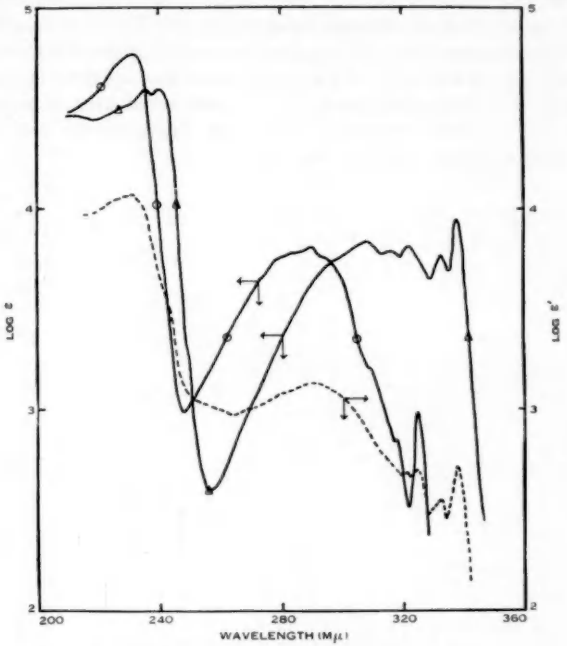


Fig. 1.—Ultraviolet absorption spectra in *iso*-octane.
○—○ Cadalene.
△—△ 5-Hydroxycadalene.
– – – – Dehydrogenation products of δ-cadinene diepoxide.

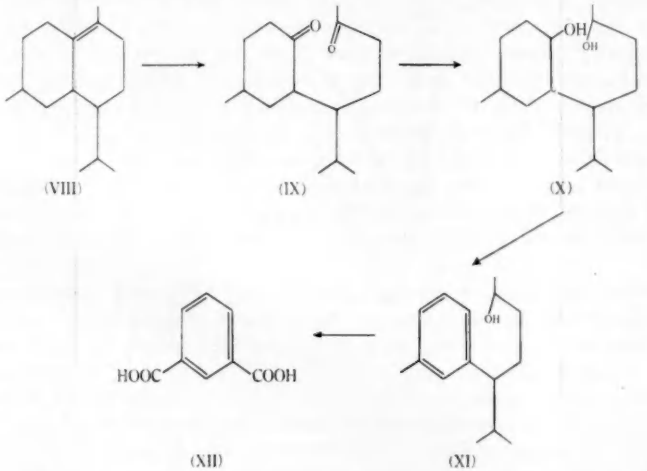
The production of 5-hydroxycadalene by dehydrogenation of the diepoxide necessitates a double bond in either the 4,5- or the 5,6-positions of dysoxylonene but the latter position is incompatible with the production of (±)-cadinene dihydrochloride and the absence of conjugation with the 10(1)-double bond (see Section IV). These results then constitute a proof of the 4,5-position of one double bond in dysoxylonene.

IV. Dihydrodysoxylonene

A direct proof of the 10(1)-situation of the second double bond was made possible by selective hydrogenation of the 4,5-double bond by Raney nickel in ethanol to yield 4,5-dihydrodysoxylonene. Both double bonds were

hydrogenated without a break in rate by Adams's catalyst and by 10 per cent. palladized charcoal.

The purity of the crude hemihydrogenation product was limited by the known impurity of the starting material, so that fractional distillation was used to obtain acceptable 4,5-dihydrodysoxylonene. Even so, a study of the physical constants (see Table 4) shows that the fractions within the boiling range 126–127·5 °C/10 mm, contained at least three substances, one being a minor constituent only. The two major components are likely to be stereoisomers differing in the configuration of the 4-methyl group. Since the infra-red spectra demonstrated the absence of all but tetrasubstituted double bonds, and since other possible sites for a tetrasubstituted double bond do not permit the production of (±)-cadinene dihydrochloride from dysoxylonene, the structure of 4,5-dihydrodysoxylonene can only be VIII.



(VIII) (IX) (X) (XI) (XII)

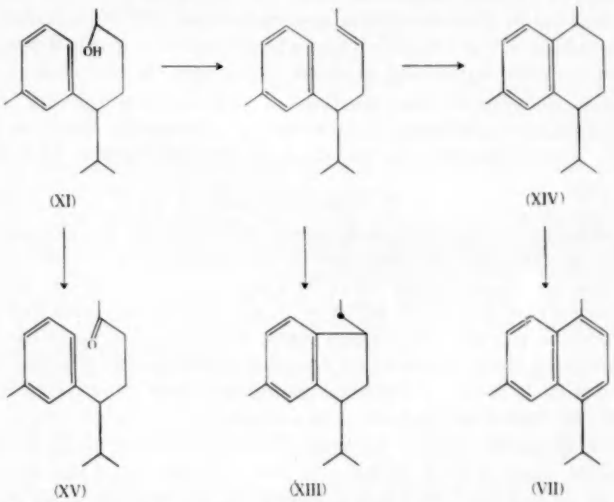
Dihydrodysoxylonene was examined by the method of Long and Neuzil (1955), who distinguished the various types of olefins by the position of the absorption maximum of the olefin-iodine complex. With increasing substitution the maximum moves from 275 mμ for $R.CH{:}CH_2$ towards the visible, three aliphatic tetrasubstituted olefins showing $\lambda_{max.}$ 337 mμ. The position (355 mμ) of the maximum (ε' 19·6) for dihydrodysoxylonene proved to be anomalous, being outside the range of wavelengths reported by Long and Neuzil.

Further confirmation of the 10(1)-double bond position was sought in carrying out the reaction scheme VIII→XII, in that *iso*phthalic acid could not result from the severance of any other tetrasubstituted bond in the cadinene skeleton.

The mono-olefin (VIII) was ozonized and the ozonide decomposed with hydrogen peroxide in acetic acid. An aldehyde-free, neutral oil comprised over 90 per cent. of products, a further indication of the tetrasubstitution of the

olefinic bond. Chromatography on alumina yielded the diketone (IX) as an oil. The only derivative obtainable as a solid, the bisdinitrophenylhydrazone, melted over a wide range. The diketone was reduced to the diol (X) to minimize the formation of phenols and to eliminate self-condensation during the dehydrogenation.

A small-scale dehydrogenation of the diol with 10 per cent. palladized charcoal commenced gas evolution at 265 °C, the yield being only 62 per cent. of that calculated for 2 moles of hydrogen. Steam distillation yielded a distillate with predominantly benzenoid-type absorption. A preparative dehydrogenation yielded a pale green oil which after being freed from a trace of phenols, was distilled under reduced pressure into a number of fractions.



(XI) (XIV)

(XV) (XIII) (VII)

The fraction of narrowest boiling range, b.p. *c.* 128 °C/3 mm, analysed satisfactorily for XI, $C_{15}H_{24}O$, for which the observed boiling point would be appropriate. Nevertheless, the fraction was undoubtedly a complex mixture since gas chromatography indicated four major constituents, one of which was recognized as cadalene. *iso*Phthalic acid was obtained (21% w/w yield of crude acid) from the fraction by permanganate oxidation.

A rational interpretation of the composition of this fraction necessitates ascribing the shoulder at 285–292 mμ and the peak at 325 mμ in the ultraviolet absorption spectrum to the presence of cadalene, 8 per cent. of which would satisfactorily reproduce the spectrum above 285 mμ. The very sharp peak at 279 mμ requires the presence of a β-alkyl tetralin or more probably a β-alkyl indan, which could be XIII. An alternative interpretation that this peak is due to phenolic material is ruled out by the lack of equally strong absorption at 279 and 286 mμ as shown by 2,4-xylenol (Friedel and Orchin 1951). This indan could arise from the dehydration product of XI, which by alternative

cyclizations could yield the indan (XIII), and the tetralin, calamene (XIV), which would be further dehydrogenated to cadalene. Assuming the ultraviolet spectrum of XIII to be identical with that of 1,6-dimethylindan (American Petroleum Institute Project No. 44, Collection No. 614), the addition of the spectrum due to 16 per cent. of the indan to that of the cadalene gives a satisfactory representation of the observed spectrum about 277 mμ. To reproduce the strong broad peak at 262 mμ, the further addition of about 60 per cent. of XI and XV is sufficient, the spectra of these substances being taken as identical with that of *m*-cymene (A.P.I. Collection No. 167). The presence of both X and XV is consistent with the infra-red absorption spectrum which shows both carbonyl and hydroxyl absorption and the total quantity is satisfactorily related to the observed yield of *iso*phthalic acid. The residual difference between the observed and the totalled absorption spectra rises roughly linearly from $E^{1\%}_{1\,cm}$ 3 at 270 mμ to $E^{1\%}_{1\,cm}$ 130 at 245 mμ and may be attributed to residual non-aromatic oxygenated material amounting to about 15 per cent. of the whole.

Another dehydrogenation fraction of b.p. 92–100 °C/3 mm resembled 1,6-dimethylindan considerably more closely in ultraviolet spectrum than did fraction 4. *iso*Phthalic acid was not obtained from this fraction by oxidation.

V. Dysoxylonene

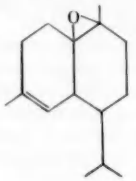
The isolation of *iso*phthalic acid proves independently the site of the tetrasubstituted ethylenic link in dysoxylonene and confirms the cadina-4,10(1)-diene structure for this hydrocarbon. The coincidence of the physical constants of dysoxylonene and of δ-cadinene (d_4^{20} 0·9175, n_D^{20} 1·5086, $[\alpha]_D$ +94°) as described in the literature (Herout and Sykora 1958) is satisfactory since the optically active δ-cadinene from citronella oil contained considerably less (log ε 2·55 at 246 mμ) of what is probably the same conjugated diene impurity as is found in the δ-cadinene from rosewood oil.

Despite the zero rotation of dysoxylonene diepoxide, hydrochloride, and hydrobromide, there is some evidence in the laevorotation of the dysoxylonene fractions 40–60 and more convincing evidence in the dextrorotation of the pure dihydrodysoxylonene fractions and in the laevorotation of the crude dehydrogenation products that the dysoxylonene is not exactly racemic. Dysoxylonene is then δ-cadinene of low optical purity from which optically inactive derivatives may be readily isolated. Although cadinenes yielding (+)-cadinene dihydrochloride are but rarely found (e.g. Birch 1953) by contrast to those yielding the (−)-dihydrochloride, the existence in separate plants of the appropriate enzyme systems for the production of the enantiomorphs renders it statistically likely that some few plants will have both systems and will produce cadinenes at least partially racemic, as frequently happens with the monoterpenes, α-pinene, limonene, etc. The production of a pure racemate however by the enzyme systems of a plant would necessarily be an extraordinary coincidence. We agree with Hellyer and McKern (1956) that the name dysoxylonene should be abandoned.

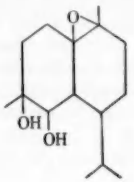
A monoepoxide of δ-cadinene is readily prepared by the use of peracids in ether since in this solvent the second double bond shows little tendency to epoxidation. The monoepoxide is an oil of b.p. 123–124 °C/5 mm and n_D^{25} 1·4986

for which the structure XVI was demonstrated by dehydrogenation of a small sample and examination of the ultraviolet absorption spectrum of the steam volatile products. 5-Hydroxycadalene was absent, cadalene alone being formed and identified as the picrate.

The epoxydiol obtained from the partial hydrolysis of the diepoxide and also formed during dehydrogenation of the diepoxide probably has structure XVII. A microsample heated either alone or with palladized charcoal at 265 °C yielded crystals in the refluxing liquid above the surface of the bath. The crystals



(XVI)



(XVII)

reformed repeatedly after being melted down into the reaction vessel containing the palladized charcoal, so that it was not possible to complete the dehydrogenation in 1½ hr. The yield of aromatic material was lower than from the dehydrogenation of the diepoxide and the naphthol to hydrocarbon ratio was also lower. This behaviour suggests that the 4,5-epoxide group has been replaced by two hydroxyls, equatorially arranged in view of the observed stability, and that the formation of this epoxydiol is not a step in the principal route by which diepoxide is dehydrogenated to aromatics.

VI. Experimental

Melting points are not corrected. Analyses are by Mr. J. Kriaciunas, University of Queensland, and Dr. K. W. Zimmermann, C.S.I.R.O. and the University of Melbourne Microanalytical Laboratory.

(*a*) *Isolation of the Wood Oil.*—Commercial planks of *D. frazeranum*, sawn from one log, from the Mt. Glorious area, were reduced to shavings which were steam distilled with cohobation of the aqueous layer to yield an intensely blue oil (2·2% yield, d_4^{25} 0·9333, n_D^{25} 1·5100).

(*b*) *Fractional Distillation of the Wood Oil.*—From preliminary distillation of 2800 ml of wood oil was obtained 910 ml of oil of boiling range 123–140 °C/10 mm. This was fractionated in a Podbielniak hypercal still (6 ft of Heligrid packing of about 100 theoretical plate equivalents) under a still head pressure of 10 mm using a reflux ratio of 40–50. Continuous operation was required, the distillation taking about 3 weeks for completion. The distillate was delivered into an automatic fraction collector which cut the fractions to constant volume (*c.* 10 ml). Table 1 presents the physical constants for some of the fractions obtained from the distillation. Because of the difficult separations involved, the physical properties must change gradually and measurement of the properties of each fraction was considered unnecessary.

(*c*) *Dysoxylonene.*—Fractions 40–60, except No. 50, were combined to form a bulk stock (185 ml) of "dysoxylonene concentrate" so-called. The ultraviolet light absorption of this concentrate showed ε 4650 at 220 mμ, $\varepsilon_{min.}$ 910 at 236 mμ, $\varepsilon_{max.}$ 2100 at 247 mμ, and ε 1330 at 260 mμ. Infra-red bands were observed at 1649 cm^{-1} (medium) and 887 cm^{-1} (strong) which may be assigned to $R_1R_2C:CH_2$ and bands at 1668 cm^{-1} (weak) and 840 cm^{-1} (strong) due to ethylenic trisubstitution. Absorption indicative of other types of ethylenic substitution was absent.

(*d*) *Dysoxylonene Dihydrochloride.*—Portions (1 g) of various fractions in ether solution were treated in a freezing mixture with hydrogen chloride gas. The yields of dysoxylonene dihydrochloride are noted in Table 1. Recrystallization from ethyl acetate yielded colourless needles, m.p. 105–106 °C, $[\alpha]_D^{25}$ ±0° ($CHCl_3$).

(*e*) *Dysoxylonene Dihydrobromide.*—Dysoxylonene concentrate (2·0 g) in anhydrous ether (1 ml) was saturated with hydrogen bromide gas. Crystals, m.p. 123–124 °C (yield 1·40 g), separated and were recrystallized from ethyl acetate as colourless needles, m.p. 125 °C, $[\alpha]_D$ ±0·0° (*c*, 2·2 in $CHCl_3$) (Found: C, 49·3; H, 7·4%. Calc. for $C_{15}H_{26}Br_2$: C, 49·2; H, 7·2%).

(*f*) *Ozonolysis for Formaldehyde.*—Dysoxylonene concentrate (207 mg) was ozonized (100% excess of O_3) in propionic acid (1 ml) and the issuing gases were passed through an ice-cold solution of 2,4-dinitrophenylhydrazine in 2N HCl causing a precipitate to form. The ozonolysis mixture was diluted with acetic acid (5 ml) and zinc dust (½ g) was added. After 30 min, the suspension was brought to the boil, the distillate being passed into a further 20 ml of dinitrophenylhydrazine solution. The combined orange precipitates (20·6 mg) were filtered and after three crystallizations had m.p. 163–165 °C, not depressed by admixture with formaldehyde dinitrophenylhydrazone.

Under similar conditions, aromadendrene gave 60% of the theoretical yield of hydrazone and β-pinene gave 62%, the latter yield being reduced to 45% by addition of *cyclo*hexene to the β-pinene before ozonolysis.

(*g*) *Peracid Titrations.*—Dysoxylonene concentrate (1·017 g) in a chloroform solution (250 ml) of perbenzoic acid (2·72 g) at 0 °C consumed peracid equivalent to 1·30 double bonds in 11 min, 1·80 in 20 min, and 1·94 in 60 and 78 min. Dysoxylonene concentrate (1·003 g) in an ethereal solution containing a little chloroform and perbenzoic acid (3·20 g) at 0 °C, consumed peracid equivalent to 0·145 double bonds in 5 min, 0·295 in 30 min, 0·46 in 134 min, and 0·70 in 26¾ hr.

(*h*) *Isolation of Dysoxylonene Diepoxide.*—Dysoxylonene concentrate (5·5 ml) in ether (200 ml) containing excess monoperphthalic acid at 0 °C, had consumed peracid equivalent to 1·89 double bonds after 100 hr. The ethereal solution was then washed with saturated sodium bicarbonate solution and with water, dried and evaporated leaving a residue (5·56 g) of pale yellow oil.

TABLE 2

CHROMATOGRAPHY OF DIEPOXIDE PREPARATION

Fraction	Eluant	Volume (ml)	Products	Weight (g)
1	Light petroleum	100	—	
2		700	Yellow mobile oil	1·97
3		150	—	
4	Benzene	550	Crystals	1·32
5		450	—	
6	Benzene/ chloroform	500	Viscous oil	0·99

The yellow oil (on seeding) deposited colourless needles (1·0 g) of dysoxylonene diepoxide, which after recrystallization from light petroleum, had m.p. 86–87 °C, $[\alpha]_D^{25}$ ±0·0° (*c*, 6 in light petroleum) (Found: C, 76·2; H, 10·2%. Calc. for $C_{15}H_{24}O_2$: C, 76·2; H, 10·2%).

The mother liquors from the crystalline diepoxide were chromatographed on alumina (100 g) as indicated in Table 2, to yield three principal bands indicated below and several minor bands.

The material of fraction 4 consisted essentially of dysoxylonene diepoxide while that of fraction 2 was largely unchanged sesquiterpene and monoepoxide. Fraction 6 showed strong

hydroxyl absorption at 3460 cm^{-1} and is probably largely impure epoxydiol, from which however no crystals could be obtained.

(*i*) *Action of Methyl Magnesium Iodide on Dysoxylonene Diepoxide.*—Diepoxide (514 mg) in dry ether was added to the Grignard complex prepared from methyl iodide (6 g) and magnesium (1 g) in ether. The reaction mixture was refluxed with stirring for 15 hr and worked up with ammonium chloride solution in the usual way. The pale brown oily residue was chromatographed on alumina (20 g). A small band (30 mg) was removed by eluting with benzene/light petroleum and yielded crystalline dysoxylonene diepoxide (12 mg) after seeding. A second band (0·43 g) was eluted with benzene and was isolated as a gum (Found: C, 71·3, 71·0; H, 10·3, 10·7%. Calc. for $C_{16}H_{20}O_3$: C, 71·1; H, 11·1%). The gum showed strong hydroxyl absorption but no carbonyl absorption in the infra-red and gave a positive periodate test for α-glycol.

(*j*) *Action of* p-*Toluenesulphonic Acid on Dysoxylonene Diepoxide.*—(i) The diepoxide (19·4 mg) was refluxed in ethanol (100 ml) containing 1·08 g of *p*-toluenesulphonic acid under a nitrogen atmosphere. Aliquots were withdrawn at intervals, diluted with *iso*-octane, washed and dried in the usual manner, and examined in a Beckman DU spectrophotometer, allowance being made for absorption developed in a blank experiment without diepoxide. After 40 hr, the ultraviolet absorption curve showed end-absorption at 220 mμ (ε 6000), a minimum at 244 mμ (ε 2050), and a broad peak (ε 2680) at 272 mμ.

(ii) Diepoxide (1 g) in ethanol (100 ml) containing *p*-toluenesulphonic acid (1 g) was refluxed under nitrogen for 64 hr. The ethanol was partly removed by distillation and the diluted residue was extracted with *iso*-octane. Removal of the *iso*-octane left 0·55 g of pale brown oil of $E^{1\%}_{1\,cm}$ 173 at 271 mμ (max.) in *iso*-octane. The Gibbs test on this material was strongly positive but the ultraviolet absorption was not significantly affected by extraction with Claisen's alkali. Hydrogenation of the material with Adams's catalyst led to the absorption of about 0·5 mole of hydrogen (assumed mol. wt., 200) and reversion to a benzenoid-type absorption spectrum with $E^{1\%}_{1\,cm}$ 26 at 278 mμ. The aqueous alcoholic layer from the partition with *iso*-octane was extracted with ether, which was worked up in the usual way to yield a residue (0·49 g) from which colourless crystals (200 mg) were separated by filtration. The *epoxydiol*, m.p. 186·5–187 °C (Found: C, 70·5; H, 10·3%. Calc. for $C_{15}H_{26}O_3$: C, 70·8, 10·3%) formed prisms from benzene/*cyclo*-hexane (2 : 1) and showed no characteristic chromophore in the ultraviolet region but strong hydroxyl absorption in the infra-red. The mother liquor from the epoxydiol contained a pale brown oil $E^{1\%}_{1\,cm}$ 11 at 271 mμ (max.) in ethanol and gave a positive Gibbs test.

(*k*) *Dehydrogenation of Dysoxylonene Diepoxide.*—(i) Dysoxylonene diepoxide (9·7 mg) and 10% palladized charcoal (9·2 mg) were heated in a bath at 265 °C for 1 hr under nitrogen in a small test tube drawn out to form a reflux condenser. The reaction vessel after being cleaned externally was broken in a flask containing water (50 ml) and ultraviolet quality *iso*-octane (*c.* 10 ml). An oil trap and reflux condenser were mounted and the water was boiled for 1 hr. (The steam-distillation apparatus was cleaned beforehand so that no significant ultraviolet absorption collected in the *iso*-octane layer during a blank distillation without sample.) The volume (9·0 ml) of the *iso*-octane layer in the oil trap was noted after the apparatus had cooled to room temperature. An aliquot (5 ml) of the *iso*-octane was withdrawn and diluted to 25 ml (solution A) for examination of the ultraviolet spectrum in the range 350 to 250 mμ. A 2 ml sample of this solution diluted to 25 ml (solution B) proved suitable for the 250 to 220 mμ range. The following optical densities (mμ) were observed for solution A: 0·470 at 338 (max.); 0·325 at 332 (max.); 0·455 at 325 (max.); 0·435 at 322 (max.); 1·16 at 296·5; 1·28 at 290 (max.); and 1·03 at 250·5. Solution B showed a peak optical density of 0·86 at 231 mμ (max.) and 0·715 at 235·5 mμ.

(ii) The diepoxide (1·0 g) with 10% palladized charcoal (1·0 g) was heated to 265 °C for 1 hr under nitrogen. After dilution with light petroleum and filtration to remove the catalyst, the reaction mixture was allowed to stand overnight. Colourless crystals (101 mg) formed and were removed by filtration. Recrystallization from light petroleum/benzene yielded prisms, m.p. 186 °C (Found: C, 70·5; H, 10·0%) not depressed by admixture with the epoxydiol obtained from the diepoxide by the action of *p*-toluenesulphonic acid in ethanol.

The mother liquor of the epoxydiol was chromatographed on alumina (200 g ; grade IV) as indicated in Table 3. The eluant was collected in 5 ml fractions, each of which was evaporated separately and the residue weighed, revealing that the peak of band I was separate and distinct from that of band II.

Band I yielded cadalene picrate, m.p. and mixed m.p. 115 °C. A portion (60 mg) of band II was warmed with picric acid (40 mg) in the minimum amount of ethanol to yield dark purple crystals (48 mg) of 5-hydroxycadalene picrate, m.p. 119–126 °C. Recrystallization yielded needles, m.p. 131 °C, not depressed by admixture of authentic 5-hydroxycadalene picrate, m.p. 132 °C. Similarly 62 mg of band II from another similar dehydrogenation yielded 73 mg of 5-hydroxycadalene trinitrobenzolate, m.p. 132 °C, from ethanol and not depressed by an authentic sample. Taking into account the purity of bands I and II as indicated by their ultraviolet absorption and the yields of derivatives, the yields of cadalene and hydroxycadalene are approximately 21 and 6% respectively.

TABLE 3

CHROMATOGRAPHY OF EPOXIDE DEHYDROGENATION PRODUCTS

Band	Fractions	Eluant	Volume (×5 ml)	Weight (g)
	1–8	*iso*-Octane	8	—
I	–15		7	0·26
II	–22	*iso*-Octane	7	0·11
	–40		19	—
III	–50	Ethanol	10	0·48

(*l*) *Dysoxylonene Monoepoxide.*—Dysoxylonene concentrate (10 g) was allowed to react at 0 °C in ether for 48 hr with a quantity of monoperphthalic acid equivalent to one double bond. The reaction mixture was then washed with sodium bicarbonate solution and worked up in the usual way. The residue was fractionated in a small still with 20 cm of Bower Cooke packing. After the elimination of some unchanged sesquiterpene a number of monepoxide fractions were obtained. Selected as probably the purest, was the fraction, b.p. 123–124 °C/5 mm, n_D^{25} 1·4986 (Found : C, 81·8 ; H, 10·9%. Calc. for $C_{15}H_{24}O$: C, 81·8 ; H, 11·0%). This fraction showed no carbonyl or hydroxyl absorption in the infra-red.

(*m*) *Dehydrogenation of Dysoxylonene Monoepoxide.*—The epoxide (115·5 mg) was heated under nitrogen with 114 mg of 10% palladized charcoal at 265 °C for 1 hr. The products were worked up by steam distillation as described in Section VI (*j*) (i). The absorption curve of the *iso*-octane distillate was identical with that of cadalene. The presence of cadalene was confirmed by the isolation of the picrate, m.p. and mixed m.p. 115 °C.

(*n*) *Dehydrogenation of the Epoxydiol.*—Epoxydiol, m.p. 186 °C (4·7 mg), was dehydrogenated with 10 mg of palladized charcoal as described in Section VI (*j*) (i). Prismatic crystals formed immediately in the condensation zone and persisted through the dehydrogenation period of 1½ hr despite occasional melting down. The dehydrogenation products were worked up in the usual way except that the *iso*-octane solution of steam volatile substances (11·3 ml) was examined without dilution. The solution had an optical density (mμ) of 0·33 at 339 (max.) ; 0·195 at 335 (min.) ; 0·24 at 333 (max.) ; 0·42 at 325 (max.) ; 1·32 at 296·5 ; 1·51 at 290 (max.) ; and 1·025 at 250·5.

(*o*) *Hydrogenation of Dysoxylonene.*—(i) *Adams's Catalyst.* Dysoxylonene concentrate (393 mg) in 3 ml of glacial acetic acid with 47·9 mg of Adams's catalyst absorbed hydrogen corresponding to the saturation of 2·02 double bonds before absorption ceased.

(ii) *Palladized Charcoal.* Dysoxylonene concentrate (1140 mg) with 42·5 mg of 10% palladized charcoal in 3 ml of acetic acid absorbed hydrogen corresponding to 1·94 double bonds.

(iii) *Raney Nickel.* Dysoxylonene concentrate hydrogenated at room temperature and pressure in ethanol in the presence of freshly prepared Raney nickel, absorbed hydrogen corresponding to 1·04 double bonds whereupon absorption virtually ceased. In all, 32 g of dysoxylonene concentrate was hydrogenated in this way to give 32·9 g of partially hydrogenated hydrocarbon.

(*p*) *Fractional Distillation of Crude Dihydrodysoxylonene.*—The crude hydrocarbon was fractionated at 10 mm pressure through a still with 1 m of Bower Cooke type packing (*c.* 55 plate equivalents) using a reflux ratio of 25, to yield the fractions listed in Table 4.

Fractions 2 to 6 showed end-absorption only in the ultraviolet region and their infra-red absorption spectra did not show bands indicative of di- or trisubstituted ethylenic bonds.

TABLE 4

DISTILLATION FRACTIONS FROM CRUDE DIHYDRODYSOXYLONENE

Fraction	Boiling Point (°C/10 mm)	Volume (ml)	d_4^{25}	n_D^{25}	α_D^{25} (10 cm hom.)	C* (%)	H* (%)
1	119–125	4·5	0·8879	1·4858	+2·2°		
2	–126·5	4·5	0·8855	1·4841	+2·4°	87·3	12·5
3	–126·5	4·5	0·8827	1·4869	+1·9°		
4	–126·5	4·5	0·8891	1·4887	+1·3°	87·1	12·5
5	–127	4·5	0·8920	1·4887	+1·0°		
6	–127·5	4·5	0·8953	1·4909	+1·1°		
7	–133	3·0	0·9104	1·5012	+6·6°	88·4	11·9

* Calc. for $C_{15}H_{26}$: C, 87·3; H, 12·7%. Calc. for $C_{15}H_{24}$: C, 88·2; H, 11·8%.

(*q*) *Ozonolysis of Dihydrodysoxylonene.*—(i) Dihydrodysoxylonene (1·00 g) was suspended in glacial acetic acid (10 ml) and ozone was passed to a calculated excess of 20%. Hydrogen peroxide solution (4 ml; 30%) was added and the reaction mixture was allowed to stand overnight and then added dropwise to boiling water (50 ml). After 30 min refluxing, the solution was extracted with ether, which was then washed with sodium carbonate solution, with water, and dried. Evaporation of the ether yielded 0·88 g of neutral oil.

The neutral oil was chromatographed on alumina using light petroleum/benzene and chloroform as eluants to yield three bands. The second and principal band, eluted with benzene, contained 0·58 g of yellowish oily *diketone* (Found: C, 76·1; H, 10·9%. Calc. for $C_{15}H_{26}O_2$: C, 75·6; H, 11·0%), showing strong absorption at 1705 cm^{-1}, and other bands at 3465 (very weak), 1240, 1175, and 923 cm^{-1}. The Schiff's test was negative. The diketone gave iodoform (31% yield) by Fuson and Tulloch's procedure.

Treated with Brady's reagent, the diketone yielded a *bis-2,4-dinitrophenylhydrazone* as an amorphous yellow powder, m.p. 79–85 °C, from ethanol (Found: C, 53·5; H, 5·6; N, 17·2%. Calc. for $C_{27}H_{34}N_8O_8$: C, 54·2; H, 5·7; N, 18·7%). Chromatography on alumina and keiselguhr–bentonite failed to produce a sharper melting product. Attempts to prepare the semicarbazone, *p*-nitrophenylhydrazone, and oxime yielded only oils.

(ii) Dihydrodysoxylonene (23·5 g) in glacial acetic acid (200 ml) was ozonized and oxidized with hydrogen peroxide as in Section VI (*q*) (i). Washing with sodium carbonate solution removed 2·3 g of acids and left 23·8 g of neutral oil, negative to Schiff's reagent and showing intense carbonyl absorption at 1705 cm^{-1}.

(*r*) *Reduction of the Diketone* (*IX*).—The crude diketone (23·8 g) in ether (50 ml) was added to a slurry of lithium aluminium hydride (5·86 g) in ether with stirring. After refluxing for a further 2 hr, the reaction mixture was worked up in the usual way to yield 19·1 g of colourless oily *diol* (Found: C, 73·0; H, 11·9%. Calc. for $C_{15}H_{30}O_2$: C, 74·3; H, 12·5%), showing intense hydroxyl absorption at 3320 cm^{-1} and feeble carbonyl (?) band at 1715 cm^{-1}.

(*s*) *Dehydrogenation of the Diol* (*X*).—(i) Diol (174 mg) was heated with 10% palladized charcoal (38 mg) under a reflux condenser connected to a nitrometer. Gas evolution indicated that dehydrogenation commenced as the temperature of the heating bath approached 265 °C. Raising the temperature eventually to 320 °C yielded 15·5 ml of evolved gas, the theoretical hydrogen evolution (2 moles hydrogen/mole diol) being 25 ml under the conditions of the experiment. The reaction mixture was diluted with *iso*-octane and steam distilled under an oil trap for 3 hr. The *iso*-octane solution showed characteristic benzenoid absorption with peaks at 270, 273, 279 mμ, a very small peak at 325 mμ, and inflections at 253, 265, and 290 mμ. The spectrum of the solution was scarcely affected by repeated extraction with 5% sodium hydroxide solution.

(ii) Nearly 16 g of diol was dehydrogenated with 10% palladized charcoal under similar conditions to Section V (*s*) (i). The products were recovered by steam distillation, forming 10·0 ml of pale green oil, n_D^{25} 1·4897, d_4^{25} 0·9251, α_D −2·3° (10 cm hom.). Extraction with aqueous alkali removed *c.* 20 mg of phenolic material. Of the remainder, 5·9 g was distilled under 3 mm pressure in a small still with 20 cm of Bower Cooke packing, to give the fractions listed in Table 5.

TABLE 5

DISTILLATE FRACTIONS FROM DEHYDROGENATION OF DIOL (X)

Fraction	Boiling Point (°C/3 mm)	Weight (g)	n_D^{25}	C* (%)	H* (%)
1	92–100	0·45	1·4795	83·6	12·2
2	–108	1·97	1·4820	84·5	12·4
3	–128	0·88	1·4892		
4	–128	1·07	1·4964	81·1	11·3
5	–142	0·81	1·4990		
Residue	142	0·62	—		

* Calc. for $C_{15}H_{18}$: C, 90·8; H, 9·2%. Calc. for $C_{15}H_{22}$: C, 89·0; H, 11·0%. Calc. for $C_{15}H_{24}O$: C, 81·8; H, 11·0%.

The following optical density values (mμ) were observed for an *iso*-octane solution containing 238 mg of fraction 4/litre: 0·595 at 249 (min.); 0·620 at 253 (max.); 0·58 at 257 (min.); 0·803 at 262 (max.); 0·755 at 266 (min.); 0·88 at 279 (max.); 0·66 at 287–292 (br. shoulder); 0·070 at 322 (min.); and 0·10 at 325 (max.).

(*t*) *Oxidation of Dehydrogenation Product to* iso*Phthalic Acid.*—A portion (514 mg) of fraction 4 of the dehydrogenation products of the diol (X) was added to an aqueous solution (150 ml) of potassium permanganate (7·5 g) and sodium hydroxide (0·18 g). The suspension was refluxed for 3 hr, acidified after cooling, and refluxed again for 0·5 hr. The manganese dioxide was dissolved by the passage of sulphur dioxide and the organic acids were extracted with ether. From the ether, a crystalline acid, m.p. 318–322 °C (110 mg), was recovered by washing with alkali and working up in the usual way. The neutral products consisted of an oil (100 mg) which was not further examined.

Heating the acid for 18 hr at 180 °C with 25% nitric acid in a sealed ampoule raised the melting point slightly, yielding 69 mg of acid, m.p. 322–326 °C. Recrystallization from water yielded the very characteristic needles (18 mg) of *iso*phthalic acid, m.p. 334–337 °C, not changed by a further recrystallization from water. A mixture with authentic *iso*phthalic acid, m.p. 345–346 °C, melted at 338–341 °C.

The oxidation product (6·16 mg) titrated in boiling aqueous solution with 0·485N $Ba(OH)_2$, required 1·48 ml for neutralization (Found: equiv. wt., 86. Calc. for *iso*phthalic acid: 83).

The oxidation product (8·5 mg, m.p. 334–337 °C) was esterified with diazomethane to yield a crude ester, m.p. 61–63 °C, raised to m.p. 67–68 °C by one recrystallization and not depressed by admixture of authentic dimethyl *iso*phthalate of the same melting point.

(*u*) *The Ultraviolet Absorption Spectrum of Cadalene.*—Cadalene picrate, m.p. 115 °C, was prepared by the dehydrogenation of a cadinene from oil of cade. Cadalene was recovered from the picrate by chromatography on alumina and was converted to the trinitrobenzolate which was recrystallized several times. Of this trinitrobenzolate, 10·89 mg was taken up in hot *iso*-octane and chromatographed on an alumina column (2 by 1·5 cm dia.). The cadalene passed through in the first 100 ml of eluate and its spectrum was measured using a Spectracord 4000A. Light absorption in *iso*-octane: λ (mμ) 329, 325 (max.), 322 (min.), 317·5 (max.), 309 (infl.), 294 (infl.), 290 (max.), 284 (infl.), 280 (infl.), 248 (min.), 231·5 (max.), 220; ε 75, 960, 340, 700, 1640, 5700, 6420, 6030, 5700, 965, 59600, 50600. Cadalene recovered directly from the picrate gave very similar values.

(*v*) *The Ultraviolet Absorption Spectrum of 5-Hydroxycadalene.*—Samples of 5-hydroxycadalene from successive recrystallizations of the picrate gave identical spectra within experimental error. Thus 3·90 mg of picrate was taken up in hot *iso*-octane and washed through an alumina column (10 by 3 mm dia.) until 50 ml of eluant had collected. The spectrum was measured using a Spectracord 4000A and at certain wavelengths with a Beckman D.U. spectrometer. Light absorption in *iso*-octane: λ (mμ) 345, 338 (max.), 335·5 (min.), 333 (max.), 329 (min.), 325, 322 (max.), 319 (min.), 317 (max.), 313 (min.), 308 (max.), 298 (infl.), 256 (min.), 240 (max.), 238 (min.), 235·5 (max.), 220 (min.); ε 570, 8890 (Beckman DU, 8520), 4890, 5900, 4460, 5680, 6580, 5700, 6020, 5910, 6890, 5480, 400, 39200, 38000, 39200, 28000.

Isobestic points for cadalene and 5-hydroxycadalene are found at 296·5, 250·5, and 235·5 mμ; ε being 5350, 1120, and 39200 respectively.

VII. Acknowledgments

The authors wish to thank Professor V. Herout of the Institute of Organic Chemistry, Czechoslovak Academy of Sciences, for a gift of δ-cadinene dioxide, Mr. H. H. G. McKern of the Museum of Applied Arts and Sciences, Sydney, for gifts of dysoxylonene dihydrochloride and rosewood oil, and Mr. E. C. M. Grigg of this Department for infra-red spectra. One of us (R.P.H.) is indebted to the Atomic Energy Commission and General Motors-Holden Ltd. for the award of fellowships.

VIII. References

Birch, A. J. (1953).—*J. Chem. Soc.* **1953**: 715.
Campbell, W. P., and Soffer, M. D. (1942).—*J. Amer. Chem. Soc.* **64**: 417.
Friedel, R. A., and Orchin, M. (1951).—"Ultraviolet Spectra of Aromatic Compounds." p. 39. (John Wiley & Sons Inc.: New York.)
Hellyer, R. O., and McKern, H. H. G. (1956).—*Aust. J. Chem.* **9**: 547.
Herout, V., Kolos, T., and Pliva, J. (1953).—*Chem. Listy* **47**: 440.
Herout, V., and Santavy, F. (1954).—*Coll. trav. chim. Tchécosl.* **19**: 118.
Herout, V., and Sykora, V. (1958).—*Tetrahedron* **4**: 246.
Linstead, P. P., and Michellis, K. O. A. (1940).—*J. Chem. Soc.* **1940**: 1134.
Long, D. R., and Neuzil, R. W. (1955).—*Anal. Chem.* **27**: 1110.
Morton, R. A., and de Gouveia, A. J. A. (1934).—*J. Chem. Soc.* **1934**: 925.
Plattner, P. A., and Magyar, G. (1941*a*).—*Helv. Chim. Acta* **24**: 191.
Plattner, P. A., and Magyar, G. (1941*b*).—*Helv. Chim. Acta* **24**: 1163.
Ruzicka, L., Schinz, H., and Muller, P. H. (1944).—*Helv. Chim. Acta* **27**: 197.
Ruzicka, L., and Sternback, L. (1940).—*Helv. Chim. Acta* **23**: 124.
Sutherland, M. D. (1952).—*Perfum. Essent. Oil Rec.* **43**: 453.

THE ALKALOIDS OF *HELIOTROPIUM SUPINUM* L., WITH OBSERVATIONS ON VIRIDIFLORIC ACID

By H. C. Crowley* and C. C. J. Culvenor*

[*Manuscript received June* 4, 1959]

Summary

Heliotropium supinum L. contains the pyrrolizidine alkaloids supinine, heliosupine, and echinatine as major bases. Two minor alkaloids, although not obtained in a pure state, are shown to be esters of trachelanthic acid and viridifloric acid, respectively, with 7-angelylheliotridine. The *N*-oxide of 7-angelylheliotridine, also isolated from the total crude base, is thought to be an artefact.

Viridifloric acid is shown to be (—)-*erythro*-2,3-dihydroxy-4-methylpentane-3-carboxylic acid. Its melting point is markedly influenced by minute amounts of tenacious impurities. The ester of supinidine and viridifloric acid has been prepared from these component substances.

I. Introduction

Examination of the alkaloids of *Heliotropium supinum* L., an introduced weed now spread over south-eastern Australia and common around Warracknabeal, was undertaken to determine whether, like those of *H. europaeum* and *H. lasiocarpum*, they could produce chronic liver disease in animals. The plant has already been studied in the U.S.S.R., where supinine (I) (Menshikov and Gurevich 1949) and heliosupine (II) (Denisova, Menshikov, and Utkin 1953) were isolated. The latter authors determined the structure of the trihydroxy acid present esterified in heliosupine without actually isolating it. Although this acid has the same structure as macrotomic acid (Menshikov and Petrova 1952), a statement that it is identical has been questioned by Culvenor (1956) because of the possibility of stereochemical differences. The acid from heliosupine has been shown by direct comparison to be identical with the trihydroxy acid present esterified in echimidine (Culvenor 1956).

II. Isolation and Structure of Alkaloids

Extraction of total alkaloid by the usual procedure gave a mixture of at least four constituents, R_F 0·64, 0·53, 0·39, and 0·32. Proportions of the three major components varied considerably in two samples examined; the approximate amounts of each are shown in Table 1. The *N*-oxide content of these samples was low. The base of R_F 0·39 crystallized directly from the total base of the second sample and was identified as supinine by direct comparison. The base of R_F 0·53 was found to be heliosupine. Its properties, including the unusual loss of crystallinity of the picrate hydrate on removal of water, agreed well with those reported by Denisova, Menshikov, and Utkin (1953). On hydrogenolysis it gave the 2-methylbutyric ester of hydroxyheliotridane and a trihydroxy acid

* Organic Chemistry Section, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

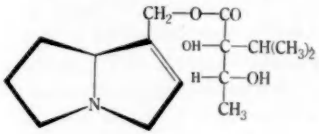
identical with echimidinic acid (III)[2]. The base of R_F 0·32 was non-crystalline like heliosupine but formed a picrolonate, m.p. 215 °C, and was identified as echinatine (IV) previously isolated from another species of the Boraginaceae, *Rindera echinata*, by Menshikov and Denisova (1953). It was hydrolysed by

TABLE 1

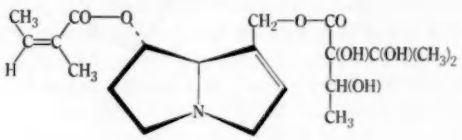
ALKALOID CONTENT OF HELIOTROPIUM SUPINUM L.

Sample	Total Base Content (% dry wt.)	Approx. Proportions of Constituents (% of total base)			
		R_F 0·64	R_F 0·53	R_F 0·39	R_F 0·32
1	0·20 (*tert.*-base, 0·17; *N*-oxide 0·03)	5	50	10	35
2	0·30	5	15	30	50

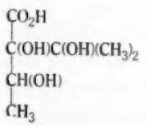
alkali to heliotridine and viridifloric acid and gave hydroxyheliotridane and viridifloric acid when subjected to hydrogenolysis. It was found surprisingly difficult to obtain a sharp and consistent melting point for viridifloric acid and since our observations account for some inconsistencies in the literature, the acid is discussed in detail later.



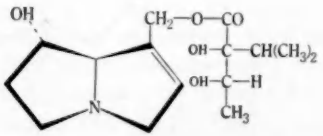
(I)



(II)



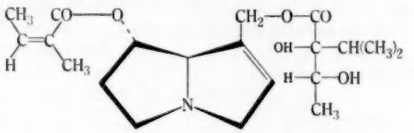
(III)



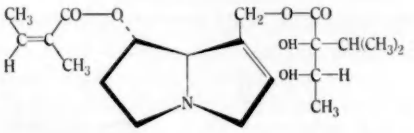
(IV)

Despite considerable effort devoted to its purification and the fact that final preparations showed only a single spot on paper chromatograms, the base of R_F 0·64 neither crystallized nor formed crystalline derivatives. Although not characterized in the ordinary sense, it was submitted to degradative study. Alkaline hydrolysis gave rise to heliotridine, angelic acid, and a crystalline acid which behaved like viridifloric acid in melting over a range of temperature. Hydrogenolysis of the base produced the 2-methylbutyric acid ester of hydroxyheliotridane and an acid similar to that obtained by hydrolysis. By chromato-

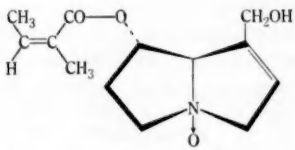
graphy on a cellulose column with butanol–ammonia solvent these acids were shown to be mixtures of trachelanthic and viridifloric acids. Since the hydrogenolysis result shows that the 7-hydroxyl group of heliotridine is esterified with angelic acid, isolation of the two acids means either that the base of R_F 0·64 is a mixture or that it has a structure in which one of the C_7 acids is esterified with an OH group of the other. A structure of this type has been proposed for lindelofamine by Labenskii and Menshikov (1948) but is ruled out in the present instance by the fact that both acids are liberated immediately by hydrogenolysis. It is concluded, therefore, that the R_F 0·64 material is a mixture of the trachelanthic (V) and the viridifloric ester (VI) of 7-angelylheliotridine. The difficulty in



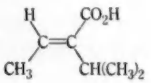
(V)



(VI)



(VII)



(VIII)

separating the mixture is due to the components being diastereoisomers. Another diastereoisomer, the trachelanthic ester of 7-angelylretronecine, is the alkaloid echiumine of *Echium plantagineum* (Culvenor 1956). Although previously recorded as R_F 0·67, this base shows the same R_F as the above material when run on the same paper sheet. Structures V and VI respectively show the absolute configuration of the pyrrolizidine nucleus but the relative configuration only of the two acids.

A third new base was isolated in very small amount as a non-crystalline *N*-oxide. Reduction of the *N*-oxide with zinc dust gave a base, R_F 0·50, which formed a picrate, m.p. 164–166 °C, thus distinguishing it from heliosupine which is of similar R_F value. When recovered from its picrate, the base was obtained

crystalline with m.p. 117–118 °C. Analysis of picrate indicated for the base a formula, $C_{13}H_{19}O_3N$, which corresponds to angelylheliotridine. It proved to be identical with 7-angelylheliotridine prepared by partial hydrolysis of lasiocarpine. The compound isolated from the crude alkaloid is therefore 7-angelylheliotridine *N*-oxide (VII). Since isolation of the *N*-oxide from a mixture of alkaloids which had been reduced (obviously incompletely) with zinc dust is a matter of chance, and since the free base is of the same R_F as heliosupine, the amount of 7-angelylheliotridine present in the total crude alkaloid is unknown. However, the amount is probably small and since it could be formed by partial hydrolysis of heliosupine it is possibly an artefact.

The preparation of supinine *N*-oxide, required for toxicological studies, and the viridifloric ester of supinidine, thought at one stage to be the base subsequently identified as 7-angelylheliotridine, are described in Section IV. The viridifloric ester is prepared from supinidine and sodium viridiflorate by the method of Culvenor, Dann, and Smith (1959).

III. Viridifloric Acid

Despite earlier isolation from two natural sources and synthesis in two laboratories, the physical constants and configurational identity of viridifloric acid have remained uncertain. The acid obtained by Menshikov (1948) from the hydrolysis of viridiflorine was reported as melting at 119–121 °C to a cloudy liquid, and, since no optical activity could be detected, as being racemic. The product of hydrolysis of echinatine was stated by Menshikov and Denisova (1953) to be identical with viridifloric acid without any physical constants being given. The Russian authors established that the acid was 2,3-dihydroxy-4-methylpentane-3-carboxylic acid. When Adams and Herz (1950) synthesized a racemic compound of this structure and of m.p. 119 °C, by permanganate hydroxylation of 2-methylpent-3-ene-3-carboxylic acid (VIII) they concluded that this was viridifloric acid.

Subsequent synthesis and resolution of both possible racemates by Adams and van Duuren (1952) and by Dry and Warren (1952) corrected this assignment by showing that the racemate, m.p. 119 °C, was actually (±)-trachelanthic acid. In both laboratories, *trans*-hydroxylation of the olefine (VIII) gave a racemate of m.p. 150 °C which was resolved via the brucine salts. For the resulting enantiomers, Dry and Warren reported m.p.'s 117–119 and 118–122 °C, with no measurable optical activity. One of these they considered would be viridifloric acid. Adams and van Duuren recorded the enantiomers as m.p. 127·5 °C, $[\alpha]_D$ +1·8° (water) and m.p. 127·5 °C, $[\alpha]_D$ −1·6° (water). However, these authors were led by certain melting point observations to conclude that the optically pure acids were capable of racemization on heating in aqueous alkali and decided that natural viridifloric acid was a partially racemized enantiomer of the racemate of m.p. 150 °C. Such racemization, however, would be very difficult to explain and a similar proposal for trachelanthic acid has already been disproved (Culvenor 1954).

Our present observations on viridifloric acid from echinatine clear up these uncertainties to a large extent. Since the acid was obtained by hydrogenolysis

of echinatine recovered from a picrolonate of sharp melting point there was little doubt that it was essentially an optically pure compound. When first isolated it was nicely crystalline but melted over a range of several degrees to give a cloudy liquid containing bubbles. Recrystallization from several solvents raised the upper limit of the melting range from about 117 °C to about 131 °C, but not consistently, and the melting point of a particular sample was partly dependent on the solvent used for crystallization. Final clarification of the melt occurred at about 140 °C. A melting point determination on a microscope hot-stage showed that a few crystals remained unmelted until about 140–142 °C, this being the cause of cloudiness between the main melting point and this upper temperature. The acid showed only one spot on a paper chromatogram and retained the above behaviour after chromatography on silica gel. The specific rotation, $[\alpha]_D$ $-1{\cdot}3 \pm 0{\cdot}2°$ (water), agreed well with the value given by Adams and van Duuren for their (−)-enantiomer but in view of the melting point difficulties it was thought desirable to check this indication of identity by direct comparison. Resolution of a sample of the synthetic (±)-compound kindly supplied by Professor Adams gave (+)- and (−)-compounds which both showed the peculiar melting point behaviour described above. Mixed melting points confirmed that the acid from echinatine was optically pure (−)-viridifloric acid; a mixture with the synthetic (−)-compound was unchanged in melting point while a 1 : 1 mixture with the (+)-compound melting sharply at 151 °C. The acid originally isolated from viridiflorine must also be (−)-viridifloric acid since Menshikov and Denisova reported having carried out a mixed melting point determination.

The peculiar melting point could at this stage be ascribed only to effects of solvation. Strong retention of solvent by the crystals was apparent from microanalyses. However, during later examination of the alkaloid mixture of R_F 0·64, viridifloric acid was isolated from a mixture with trachelanthic acid by chromatography on cellulose with butanol–ammonia as solvent. After crystallization from benzene, this product, although still apparently solvated, melted sharply at 142 °C. Identity with the acid from echinatine was confirmed by infra-red spectra and by mixed melting points; a mixture with the synthetic (+)-acid melted at 150 °C. Similar chromatography of the acid from echinatine and of synthetic (+)-acid also yielded crystals melting sharply at 142 °C in both instances. It must be concluded therefore that the true melting point of optically pure viridifloric acid is 142 °C and that persistent contamination with a minute amount of impurity is mainly responsible for the much lower melting point commonly observed. This amount of impurity does not affect the melting point of the racemate; an equimolar mixture of (+)- and (−)-acids of m.p. 142 °C still melted at 151 °C. Solvation may still play some part in lowering the melting point of the (+)- and (−)-acids since crystals formed from chloroform and carbon tetrachloride seemed to be consistently lower in melting point than other samples.

Epimerization or other change of viridifloric acid, either in alkali or by heating, might seem to offer an alternative explanation of at least some of the phenomena described, but in our hands, long heating of viridifloric acid in aqueous

alkali was found to leave the melting point and R_F unchanged (specific rotation could not be measured on the small amount of acid available at this stage). Heating at 136 °C for 4 hr also had no effect on the melting point. The change in alkali recorded by Adams and van Duuren is therefore considered to be a manifestation of the variable melting point of the slightly impure compound. Quasiracemate formation in mixtures of viridifloric and trachelanthic acids, also a possibility, is considered to be unlikely in view of the behaviour of mixtures of (−)-viridifloric and (+)-trachelanthic acid encountered in the course of this work.

IV. Experimental

Melting points are corrected. Microanalyses are made by the C.S.I.R.O. and University of Melbourne Microanalytical Service.

(*a*) *Paper Chromatography.*—The solvent system used for bases is the upper phase resulting from shaking *n*-butanol with an equal volume of 5% aqueous acetic acid. Acids are chromatographed with this solvent and with a water-saturated mixture (16 : 1) of *n*-butanol and conc. aqueous ammonia. The R_F values vary, mainly because of temperature changes, and values recorded for the alkaloids are corrected relative to 0·42 for heliotrine, which is run on all papers as a standard.

(*b*) *Total Base Extraction and Alkaloid Assay.*—The first sample of plant (5·8 kg) was extracted by percolation with cold ethanol and the extract concentrated. A precipitate which formed was removed and found to be sodium chloride (42 g). After complete removal of ethanol, the residue was taken up in dilute H_2SO_4. One-half of the acid extract was reduced with zinc dust and then both portions made alkaline (pH >9) with NH_3 and extracted with chloroform. Titration of aliquots of each product (after prior evaporation to remove NH_3) gave the content of tertiary bases before and after reduction. Paper chromatograms, run in butanol–acetic acid, showed spots of R_F 0·64, 0·53, 0·39, and 0·32 in both unreduced and reduced alkaloid. From visual appraisal of the relative sizes of the spots, and the titration figures, the data given in Table 1 were estimated. All four spots on the paper chromatograms gave positive reactions for double bonds (dilute $KMnO_4$) and for α-glycol groups (periodate–starch sprays as recommended by Metzenburg and Mitchell 1954).

The second sample of plant (84 kg) was extracted with hot methanol and then treated similarly to the above except that the whole of the aqueous acid extract was reduced with zinc dust. When it became evident that the base, R_F 0·32, was not recovered effectively by chloroform extraction under the large-scale conditions, the aqueous residues were further extracted with butanol.

(*c*) *Identification of Supinine and Heliosupine.*—The sample of heliosupine required for comparison of its main esterifying acid with that of echimidine (Culvenor 1956) was isolated from reduced alkaloid of the first sample. This base (6·2 g) was subjected to partition chromatography on a 5 by 90 cm column of Pyrex glass powder (1700 g) carrying phosphate buffer of pH 8·0 (300 ml). Eluants were light petroleum–carbon tetrachloride 80 : 20 (2·5 l.), light petroleum–carbon tetrachloride 50 : 50 (2·0 l.), carbon tetrachloride (2·0 l.), carbon tetrachloride–chloroform 60 : 40 (3·5 l.), and chloroform (6·0 l.), collected in 50 ml fractions. Fractions 21–50 contained only base R_F 0·64 (0·5 g), fractions 59–96 contained only base R_F 0·53 (2·6 g), fractions 116–146 were almost entirely base R_F 0·39 (0·46 g), and fractions 147–250 were predominantly base R_F 0·32 (1·85 g). The other component of the last groups of fractions was a base of R_F 0·24, not previously evident.

The base from fractions 116–146 was recrystallized from acetone to give supinine as colourless prisms, m.p. 148–149 °C, $[\alpha]_D^{21}$ −12° (*c*, 2·0 in ethanol). A mixture with supinine from *Heliotropium europaeum* melted at the same temperature.

Base from fractions 59–96 was treated in ethanol with a small excess of picric acid and the solution evaporated to dryness. The resulting gum was extracted with water at 80 °C. On cooling, these extracts deposited heliosupine picrate as needles, m.p. 93–96 °C. Recrystallization

from water and drying at room temperature gave yellow crystals, m.p. 103–106 °C, of a monohydrate (Found: C, 48·9; H, 5·8; N, 8·6; O, 36·6%. Calc. for $C_{20}H_{31}O_7N.C_6H_9O_7N_3.H_2O$: C, 48·5; H, 5·6; N, 8·7; O, 37·3%). When dried in a drying pistol at 56 °C, the crystals changed into a gum. Drying at 40 °C for a short period lowered the m.p. to 75–85 °C. This behaviour is that described by Denisova, Menshikov, and Utkin (1953) for heliosupine picrate and their explanation that the anhydrous picrate is non-crystalline is probably correct. Free heliosupine was recovered from the picrate as a colourless gum, $[\alpha]_D^{20}$ −4·3° (*c*, 5·10 in ethanol).

(*d*) *Fractionation of Alkaloid from the Second Plant Sample.*—The chloroform extracts of total base from the second plant sample (Section IV (*b*)) were concentrated to about 3 l. and extracted with 0·5N H_2SO_4. The solution was made alkaline and re-extracted with chloroform to give crude base as a thick gum (A; 220 g), R_F 0·64, 0·53, 0·39, 0·32 with supinine (R_F 0·39) predominating. The aqueous alkaline residue was extracted further with butanol and this extract evaporated to give a thick mobile liquid (*c.* 100 ml) obviously containing a large amount of non-basic material. This residue was taken up in dilute H_2SO_4 and the solution filtered, saturated with Na_2CO_3 and extracted many times with chloroform. The product (B; 13·9 g) was mostly of R_F 0·32 with some base of R_F 0·61. Re-extraction of the aqueous residue with butanol gave a thick liquid (2·9 g). Addition of acetone precipitated inorganic material and evaporation of the acetone left an oil (C; 1·54 g) mostly of R_F 0·61. The high R_F value indicated that this substance was mainly *N*-oxide, and this was confirmed when reduction of a test portion gave a base, R_F 0·50, readily extracted from water with chloroform.

TABLE 2

PARTITION COEFFICIENTS OF SUPININE ($c_{org.\ phase}/c_{aq.\ phase}$)

Solvent Phases	Partition Coefficient	Solvent Phases	Partition Coefficient
Ether: pH 8·0 buffer ..	0·02	Chloroform: pH 8·0 buffer ..	1·1
Ether: pH 8·9 buffer ..	0·27	Chloroform: pH 8·9 buffer ..	8·0

The butanol extract of residual base, mentioned in Section IV (*b*), was evaporated to give a thick syrup (*c.* 1·5 l.) showing a large number of spots with R_F 0·42 predominant. The syrup was taken up in water (12 l.), made alkaline to phenolphthalein, saturated with NaCl, and extracted with eight lots of chloroform to give an amber gum (D; 48 g), R_F 0·32. Since the component of R_F 0·42 was not recovered at all, *N*-oxide was again suspected. The residual aqueous solution was made 2E with respect to H_2SO_4, reduced with zinc dust, made alkaline with NH_3, and re-extracted with chloroform to give additional base as a gum (E; 21·5 g) R_F 0·32.

Fraction A crystallized when seeded with supinine. It was slurried with acetone (150 ml) and filtered to give supinine (83 g), m.p. 148–149 °C after recrystallization from acetone. Treatment of the mother liquors aimed at separating the material into a concentrate of heliosupine and a mixture of supinine and base R_F 0·32. This was facilitated by determination of the partition coefficients of supinine, shown in Table 2. Base from the mother liquors, together with another 30 g of similar constitution was dissolved in a mixture of phosphate buffer of pH 8·0 (6 l.) and ether. The aqueous phase was extracted with several further portions of ether (total, 12 l.). Evaporation of the ether gave a gum (F; 45 g) which showed R_F values 0·64 (faint), 0·53 (strong), 0·39 (trace), and was thus the desired heliosupine concentrate. Separation of the supinine and base R_F 0·32 was also achieved by making the buffer phase alkaline to phenolphthalein, extracting with carbon tetrachloride to give supinine (8·12 g) which crystallized on seeding, and then saturating with NaCl and extracting with chloroform to give base (G; 45 g) which was almost entirely of R_F 0·32.

The heliosupine concentrate, F, contained all the R_F 0·64 base, and was divided into a concentrate of the latter and heliosupine by shaking with pH 7·5 buffer (400 ml) and light

petroleum : carbon tetrachloride 80 : 20 (400 ml). The latter phase and a further portion of this solvent used to wash the aqueous phase were combined and evaporated to give a gum (H ; 14·9 g), R_F 0·64, 0·53 (about equal intensities). The buffer was then extracted with chloroform to give a gum (30·2 g), almost pure heliosupine. This product was converted into picrate and the latter crystallized from water giving yellow needles (34·2 g), m.p. 101–103 °C.

(*e*) *Isolation of Echinatine.*—Fractions B, D, E, and G contained mostly the base R_F 0·32 but some were contaminated with *N*-oxides of R_F about 0·5–0·6. These were reduced with zinc in H_2SO_4 and the base recovered as before. All concentrates were then treated with picrolonic acid in ethanol to give yellow needles, m.p. 214 °C (decomp.). Recrystallization from ethanol gave needles, m.p. 215 °C (decomp.) (Found : C, 53·4 ; H, 6·1 ; N, 12·2%. Calc. for $C_{15}H_{25}O_5N.C_{10}H_8O_8N_4$: C, 53·3 ; H, 5·9 ; N, 12·4%). For echinatine picrolonate, Menshikov and Denisova (1953) record m.p. 206 °C (uncorr.). Echinatine was recovered from the picrolonate by dissolving the latter in hot water, adding a small excess of copper sulphate, filtering off the precipitated copper picrolonate, making the filtrate alkaline, and extracting with chloroform. It formed a light amber gum, $[\alpha]_D^{22}$ +15° (*c*, 2·6 in ethanol). The total yield of crystalline picrolonate was only about one-half of that expected from the weights of the crude base fractions. A substantial amount of black tarry material resulted from the picrolonate preparation and it seems likely that either echinatine or another substance present in the concentrates is oxidized by picrolonic acid.

(*f*) *Purification of the* R_F *0·64 Base.*—A portion (6·9 g) of fraction H was chromatographed on a column of Pyrex glass powder (5 by 90 cm) bearing a pH 7·5 buffer (240 ml). Base R_F 0·64 (2·69 g) was eluted by light petroleum–carbon tetrachloride 80 : 20 (1·5 l.) and heliosupine (4·1 g) by a further quantity of this solvent (800 ml) and carbon tetrachloride (2 l.). The remainder of fraction H was treated similarly to give in all 4·1 g of base R_F 0·64 showing no other spot on a paper chromatogram. Attempts to prepare a crystalline picrate, picrolonate and *N*-oxide of the base were unsuccessful. The *N*-oxide preparation had R_F 0·72, about that expected.

(*g*) *Isolation of 7-Angelylheliotridine.*—Fraction C (see Section IV (*d*)) was reduced with zinc dust in dilute H_2SO_4 and worked up as usual to give a gum (0·94 g), R_F 0·50. This was neutralized with picric acid in ethanol, evaporated to dryness, and the product crystallized from water to give yellow needles, m.p. 149–152 °C, depressed by 30 °C on admixture with echimidine picrate (Culvenor 1956). Three recrystallizations from water and from ethanol gave *7-angelylheliotridine picrate*, m.p. 164·5–165 °C (Found : C, 49·1 ; H, 4·9 ; N, 12·0%. Calc. for $C_{13}H_{19}O_3N.C_6H_3O_7N_3$: C, 48·9 ; H, 4·8 ; N, 12·0%). A mixed melting point with authentic material (Section IV (*p*)) was undepressed. After its structure had been established recovery of the base from the picrate was effected by passage in aqueous acetone through " Dowex 1–X4 ". After seeding with the compound derived from lasiocarpine, it crystallized as needles from light petroleum, m.p. and mixed m.p. with *7-angelylheliotridine* 116–117 °C (Found : C, 65·7 ; H, 7·9 ; N, 6·0%. Calc. for $C_{13}H_{19}O_3N$: C, 65·8 ; H, 8·0 ; N, 5·9%).

(*h*) *Hydrogenolysis of Heliosupine.*—Heliosupine (0·55 g) recovered from its picrate, shaken with hydrogen and PtO_2 catalyst in dilute HCl, absorbed 3 mol. hydrogen. The solution was filtered, made alkaline, and extracted with chloroform to give a liquid base (0·32 g) mostly of R_F 0·69, but containing some bases of lower R_F. Two distillations from a bulb tube at bath temperature 65 °C/0·2 mm gave a colourless oil, $[\alpha]_D^{17}$ +0·9±1° (*c*, 1·1 in ethanol) (Found : N, 6·3%. Calc. for $C_{13}H_{23}O_2N$: N, 6·2%). The picrate formed needles from ethanol, m.p. 158–159 °C undepressed on admixture with the picrate of the α-methylbutyric ester of hydroxyheliotridane derived from lasiocarpine (Found : C, 50·5 ; H, 6·1 ; N, 12·2%. Calc. for $C_{13}H_{23}O_2N.C_6H_3O_7N_3$: C, 50·2 ; H, 5·8 ; N, 12·3%).

The aqueous residue after removal of the basic product was made just acid to Congo red with HCl, and evaporated in an air stream and finally in a desiccator over NaOH. Extraction of the residue with hot chloroform gave a clear glassy acid (0·22 g), R_F 0·54 in butanol–acetic acid. It was converted into the brucine salt, crystals from ethanol, m.p. 208–210 °C, undepressed on admixture with the brucine salt of echimidinic acid (Found : C, 63·1 ; H, 7·1 ; N, 4·9%. Calc. for $C_7H_{14}O_5.C_{23}H_{26}O_4N_2$: C, 62·9 ; H, 7·0 ; N, 4·9%). Recovered from the brucine salt, the

acid formed a glass, $[\alpha]_D^{20}$ +17·5° (*c*, 1·1 in alcohol). Echimidinic acid is recorded as having $[\alpha]_D^{20}$ +16·4° in ethanol (Culvenor 1956).

(*i*) *Hydrolysis of Echinatine.*—Echinatine (1·0 g), recovered from its picrolonate, was refluxed with barium hydroxide (2·0 g) in water (15 ml) for 2 hr, carbon dioxide passed through the solution, the barium carbonate filtered off, the filtrate acidified with HCl and extracted with ether to give a crystalline acid (0·45 g), m.p. 123–127 °C, after crystallization from ethyl acetate–light petroleum. This acid, viridifloric acid, is discussed further in Section IV (*k*).

The aqueous solution was passed through " Deacidite FF " and the filtrate evaporated to dryness under reduced pressure to give heliotridine as a gum (0·40 g) which crystallized from acetone as needles, m.p. 115–116 °C, mixed m.p. 115–116 °C, $[\alpha]_D^{20}$ +30° (*c*. 1·59 in methanol and *c*, 1·59 in ethanol) (Found: C, 62·1; H, 8·3; N, 9·0%. Calc. for $C_8H_{13}O_2N$: C, 62·0; H, 8·4; N, 9·0%).

(*j*) *Hydrogenolysis of Echinatine.*—The base (4·0 g), shaken with hydrogen and PtO_2 in dilute H_2SO_4, took up hydrogen very slowly but eventually consumed almost 2 moles. Filtering the solution and extracting with ether gave viridifloric acid (1·5 g), which formed needles, m.p. 117–119 °C, from chloroform, undepressed on admixture with the acid from the hydrolysis of echinatine (see also Section IV (*k*)). The basic product (1·5 g) was recovered by making the aqueous solution alkaline and extracting with chloroform. Only a small proportion of the base distilled from a bulb tube (at bath temperature 85–100 °C/0·1 mm) but the distillate was partially crystalline and formed needles from light petroleum, m.p. 59–62 °C, undepressed on admixture with hydroxyheliotridane, $[\alpha]_D^{20}$ −15° (*c*, 1·4 in ethanol).

(*k*) *Viridifloric Acid.*—The acid obtained from hydrolysis of echinatine (Section IV (*i*)) formed long needles from ethyl acetate–light petroleum, m.p. 123–127 °C, unaltered by drying at 80 °C *in vacuo* under which conditions it partially sublimed. After several recrystallizations, the melting point was raised to 128–132 °C; on all occasions it melted to a cloudy liquid, usually

TABLE 3

SPECIFIC ROTATION OF VIRIDIFLORIC ACID

Solvent	*c*	$[\alpha]_D^{20}$
Ethanol	2·23	−0·34°
Water	2·06	−1·3°
0·8N NaOH	2·0	−2·1°
2N NaOH	2·0	−5·0°

with bubbles appearing in the melt and the melt finally clarifying at about 136–139 °C. The melting point (understood as the early main melting temperature) did not rise consistently and sometimes fell after another crystallization. After drying at 60 °C *in vacuo* for 2 hr the sample of m.p. 128–132 °C still retained solvent (Found: C, 54·0; H, 8·9%; equiv. wt., 171. Calc. for $C_7H_{14}O_4$: C, 51·8; H, 8·7%; equiv. wt., 162). On recrystallization from chloroform the m.p. fell to 117–119 °C, unaltered by drying in a vacuum (Found: C, 49·3; H, 8·2%; hence solvated). Sublimation at 100 °C/0·5 mm gave a glassy deposit, m.p. 116–118 °C (to cloudy liquid), which analysed correctly (Found: C, 52·1; H, 8·6%).

The acid from hydrogenolysis of echinatine (Section IV (*j*)) behaved in exactly the same way showing that the observed effects were not due to decomposition or epimerization of the acid by heating with aqueous alkali. Specific rotation measurements were made on this acid in a 4-dm tube with results as shown in Table 3. Acid from both sources showed only one spot on paper chromatograms, R_F 0·68 in butanol–acetic acid, R_F 0·29 in butanol–ammonia.

Acid of m.p. 127–130 °C was converted into the brucine salt which was highly soluble in alcohol and was recrystallized from a small volume of acetone to give microneedles, m.p.

195–196 °C (Found: C, 64·2; H, 7·3; N, 5·3%. Calc. for $C_{30}H_{40}O_8N_2$: C, 64·7; H, 7·2; N, 5·0%). This agrees reasonably well with the solubility and m.p. of the (—)-acid-brucine salt reported by Adams and van Duuren.

The m.p. of the acid appeared to depend mostly on the solvent employed for crystallization. Toluene and ethyl acetate–light petroleum gave the highest m.p., the former being the most convenient. Sublimation at 90–100 °C/0·5 mm gave a powder, m.p. 118–123 °C, also forming a cloudy liquid. Heating the acid in a capillary tube at 136 °C for 4 hr or refluxing in toluene for 5 hr did not affect the m.p. showing that the acid was not being decomposed by heat. A determination of m.p. on a microscope hot-stage showed that no changes other than melting were taking place but a few crystals did persist up to 142 °C before melting. This was evidence that an impurity was present and the acid was chromatographed on a column (80 by 2·2 cm) of silica gel. All eluted fractions were unchanged except that the last one (2% of the acid applied) had m.p. 129–137 °C.

(i) *Comparison with Synthetic Acid.* A sample (0·25 g) of synthetic racemate (supplied by Professor Adams) was resolved by means of the brucine salts. Several crystallizations of the mixed salts from ethanol gave pure salt of the (+)-acid (0·37 g), m.p. 183–184 °C, which has only a low solubility in this solvent. The brucine salt of the (—)-acid which is very soluble in alcohol was obtained by concentrating the mother liquor and removing the solid which separated (0·13 g), m.p. 177–184 °C), evaporating the filtrate and crystallizing the residue from acetone (0·17 g, m.p. 190–192 °C) then from a very small amount of ethanol and finally from acetone. It formed tiny rosettes, m.p. 196–197 °C. Reworking of mother liquors gave an additional 0·14 g, m.p. 195–196 °C. Thus the brucine salt of the (—)-acid had the same properties as that of the naturally occurring acid; a mixture of the two had m.p. 195–196 °C.

The pure synthetic (+)- and (—)-acids were recovered from the brucine salts by dissolving in dilute H_2SO_4 and extracting with ether. Both showed the same m.p. behaviour as the naturally occurring acid; e.g. the (+)-acid, crystallized from ether–light petroleum sintered at 122 °C, melted at 129–132 °C to a cloudy liquid which became clear at 138 °C. Equal quantities of the (+)- and (—)-acids, mixed in ether and re-evaporated, gave the racemate, m.p. 150–151 °C (sharp, with no sign of prior sintering). The m.p. of the acid from echinatine was unaffected by admixture with synthetic (—)-acid, and raised to 150–151 °C by admixture with the synthetic (+)-acid.

(ii) *Final Purification.* The above observations appeared to show that the peculiar m.p. behaviour was a property of pure viridifloric acid. However, at a much later stage during examination of the R_F 0·64 material, it was found that chromatographic separation of viridifloric acid from trachelanthic acid on a cellulose column with butanol–ammonia solvent gave the former acid with m.p. 142 °C after crystallization from benzene. This is described in Section IV (*n*). The acid from echinatine and the synthetic (+)-acid were then chromatographed in the same way and both acids were obtained as needles from benzene, m.p. 142 °C (sharp). A mixture of the (+)- and (—)-acids of this m.p. melted at 151 °C. The small amounts of acid available precluded specific rotation measurements.

(*l*) *Hydrolysis of Base,* R_F *0·64.*—The base (1·4 g) was dissolved in ethanol (12 ml), mixed with 10% aqueous NaOH (30 ml), and refluxed for 2 hr with an air stream passing through the condenser to carry volatile carbonyl compounds into a solution of 2,4-dinitrophenylhydrazine hydrochloride. From the latter was obtained an orange precipitate (0·06 g) of m.p. 119–120 °C, raised to 126–130 °C after two crystallizations from ethanol. Chromatography in *cyclo*hexane saturated with dimethylformamide on paper impregnated with dimethylformamide (Gasparic and Vecera 1957) showed it to be a mixture of the dinitrophenylhydrazones of acetone (R_F 0·36) and acetaldehyde (R_F 0·19). In this system the R_F values are dependent on the degree of saturation of the tank space with *cyclo*hexane vapour, and paper sheets soaked in the solvent should be hung on either side of the paper being developed. The acetaldehyde may be derived from the main bases present, but the acetone must come from an impurity (possibly a trace of heliosupine).

The reaction solution was acidified with HCl and extracted with ether. The product (0·56 g) was redissolved in water, washed in alkaline solution to remove non-acidic impurities, acidified,

and extracted with light petroleum to give angelic acid (0·17 g), m.p. and mixed m.p. 43 °C. The aqueous solution was re-extracted with ether to give a pale yellow gum (0·14 g), R_F 0·67 in butanol–acetic acid. This gum was chromatographed on silica gel. Nothing was eluted by chloroform but ether eluted an acid which crystallized when stored in a desiccator: m.p. 108–111 °C. This acid is discussed further in Section IV (*n*).

The residual aqueous reaction solution was evaporated to dryness, the residue extracted with ethanol and re-evaporated, and the product taken up in water, passed through " Deacidite FF " and re-evaporated. The residue, recrystallized from acetone, gave heliotridine (0·36 g) as needles, m.p. 116–117 °C, undepressed on admixture with an authentic specimen (Found: C, 61·8; H, 8·2; N, 8·9%. Calc. for $C_8H_{13}O_2N$: C, 62·0; H, 8·4; N, 9·0%).

(*m*) *Hydrogenolysis of Base*, R_F *0·64*.—In view of the suspected impurity in the base subjected to hydrolysis, the base used for this experiment was further distributed between chloroform and 0·2N HCl (6 transfers) and chromatographed on Pyrex glass powder bearing a pH 4·0 buffer. The material hydrogenolysed (1·1 g) was a pale amber gum; shaken with hydrogen and platinum oxide in dilute HCl, it absorbed 175 ml (calc. for 3 moles; 180 ml). The solution was filtered and extracted with ether to give a crystalline acid (0·44 g), R_F 0·67 in butanol–acetic acid. Recrystallized from benzene, it formed needles, which melted at 99–111 °C to a cloudy liquid. The brucine salt had m.p. 195–200 °C when first prepared and m.p. 220–221 °C when recrystallized from ethanol. Further details are in Section IV (*n*).

The residual aqueous reaction solution was made alkaline with NaOH and extracted with chloroform to give an oil (0·50 g), R_F 0·70, which formed from ethanol, a picrate, m.p. 159–160 °C (Found: C, 50·4; H, 5·8; N, 12·0%. Calc. for $C_{19}H_{26}O_9N_4$: C, 50·2; H, 5·8; N, 12·3%). A mixture with the picrate of the α-methylbutyric ester of hydroxyheliotridane, m.p. 158–159 °C, derived from heliosupine, had m.p. 158–159 °C.

(*n*) *The Acids Derived from Base*, R_F *0·64*.—When first isolated the acid mixture obtained by hydrolysis and hydrogenolysis of the base R_F 0·64 was thought to be a single (new) acid. It showed only one spot, R_F 0·67, in butanol–acetic acid. Subsequently, acid from both sources was found to be a mixture, giving two spots, R_F 0·40, 0·29, with butanol–ammonia. As chromatography on silica gel did not effect resolution, the mixture was passed through a cellulose column in butanol–ammonia as solvent. This gave virtually complete resolution. The R_F 0·40 acid crystallized from benzene–light petroleum as tiny rosettes, m.p. 90–92 °C, undepressed on admixture with trachelanthic acid (Found: C, 52·2; H, 8·7. Calc. for $C_7H_{14}O_4$: C, 51·8; H, 8·7%). The R_F 0·29 acid, after one recrystallization from benzene, melted sharply at 140 °C, a mixture with a sample of viridifloric acid of m.p. 129–133 °C melted at 135–139 °C. A mixture with an equal weight of synthetic (+)-viridifloric acid had m.p. 149–151 °C, the melting point of the racemate. Recrystallization for analysis gave needles, m.p. 141 °C (Found: C, 54·8; H, 8·9%. Calc. for $C_7H_{14}O_4$: C, 51·8; H, 8·7%). The sample is therefore solvated as was found for the acid from echinatine (Section IV (*k*)). Any doubt that the compound was viridifloric acid was removed when viridifloric acid from other sources was obtained with the same m.p. after similar chromatographic treatment (Section IV (*k*)).

(*o*) *Ester of Supinidine and Viridifloric Acid*.—Supinidine (0·30 g) was neutralized in alcohol with HCl and the solution evaporated to dryness. The product was mixed with excess ice-cold thionyl chloride and the mixture allowed to reach room temperature and left for 1 hr. Excess thionyl chloride was removed under reduced pressure. To the residue was added an aqueous solution of viridifloric acid (0·33 g) just neutralized with NaOH. The mixture was refluxed for 2 hr, cooled, filtered, made alkaline, and extracted with chloroform to give a gum (0·15 g), R_F 0·62, 0·37 (main spot), 0·19, 0·18, 0·06. To remove the substances of low R_F, the gum was redissolved in water, made alkaline, and re-extracted with carbon tetrachloride. The product (0·05 g), R_F 0·37, did not crystallize but formed crystals with aqueous picric acid. Recrystallization from water gave needles of *viridiflorylsupinidine picrate*, m.p. 112–113 °C (Found: C, 48·4; H, 5·6; N, 10·5%. Calc. for $C_{15}H_{23}O_4N.C_6H_3O_7N_3O.5H_2O$: C, 48·4; H, 5·6; N, 10·7%).

(*p*) *7-Angelylheliotridine*.—Lasiocarpine (0·5 g) was heated at 80 °C for 1·5 hr in methanol (5 ml) and 0·09N NaOH (15 ml; 1 mole). The methanol was removed under reduced pressure, additional alkali added, and the product extracted with chloroform to give a gum (0·22 g),

R_F 0·50, which crystallized. Recrystallization from light petroleum gave prisms, m.p. 116–117 °C, $[\alpha]_D^{17}$ +10·8° (*c*, 2·0 in ethanol) (Found : C, 65·3 ; H, 8·0 ; N, 5·8%. Calc. for $C_{12}H_{19}O_2N$: C, 65·8 ; H, 8·0 ; N, 5·9%). From alcohol the base formed a picrate, m.p. 165–166 °C (Found : C, 49·4 ; H, 4·8 ; N, 11·9%. Calc. for $C_{12}H_{19}O_2N.C_6H_3O_7N_3$: C, 48·9 ; H, 4·8 ; N, 12·0%).

(*q*) *Supinine N-Oxide.*—Supinine (1 g) and hydrogen peroxide (130 vol. ; 1·5 ml) were mixed in water (2 ml) and ethanol (10 ml), the solution left for 3 days, made alkaline with NaOH, left for another 4 hr until excess peroxide was decomposed, acidified with HCl, and evaporated to dryness. The residue was extracted with ethanol and the extract re-evaporated. The product was dehydrated by evaporating a benzene solution and chromatographed on alumina. Chloroform eluted unreacted supinine (0·2 g), R_F 0·39, and chloroform–5% ethanol eluted *supinine N-oxide* (0·6 g), R_F 0·49. Recrystallization from acetone (large volume required for fairly pure material) gave the *N*-oxide as long needles, m.p. 167–168 °C (Found : C, 60·5 ; H, 8·5 ; N, 4·4%. Calc. for $C_{12}H_{23}O_2N$: C, 60·2 ; H, 8·4 ; N, 4·7%). Supinine *N*-oxide is hygroscopic in humid weather. In another preparation, chromatography on alumina was dispensed with, but thorough drying of the preparation was necessary before the *N*-oxide could be crystallized.

V. Acknowledgments

The authors are indebted to Professor R. Adams, University of Illinois, for a sample of synthetic (±)-viridifloric acid and to Mr. W. T. Parsons and Inspector E. J. Heenan of the Victorian Lands Department for assistance in obtaining plant material. They also thank Dr. A. K. Kiang, University of Malaya, for experimental assistance during a visit to these laboratories.

VI. References

Adams, R., and van Duuren, B. L. (1952).—*J. Amer. Chem. Soc.* **74** : 5349.
Adams, R., and Herz, W. (1950).—*J. Amer. Chem. Soc.* **72** : 155.
Culvenor, C. C. J. (1954).—*Aust. J. Chem.* **7** : 287.
Culvenor, C. C. J. (1956).—*Aust. J. Chem.* **9** : 512.
Culvenor, C. C. J., Dann, A. T., and Smith, L. W. (1959).—*Chem. & Ind.* **1959** : 20.
Denisova, S. I., Menshikov, G. P., and Utkin, L. M. (1953).—*Dokl. Akad. Nauk. S.S.S.R.* **93** : 59.
Dry, L. J., and Warren, F. L. (1952).—*J. Chem. Soc.* **1952** : 3445.
Gasparic, J., and Vecera, M. (1957).—*Coll. Trav. chim. Tchécosl.* **22** : 1426.
Labenskii, A. S., and Menshikov, G. P. (1948).—*J. Gen. Chem. Moscow* **18** : 1836.
Menshikov, G. P. (1948).—*J. Gen. Chem. Moscow* **18** : 1736.
Menshikov, G. P., and Denisova, S. I. (1953).—*Sborn. Stat. Obshch. Khim.* **2** : 1458.
Menshikov, G. P., and Gurevich, E. L. (1949).—*J. Gen. Chem. Moscow* **19** : 1382.
Menshikov, G. P., and Petrova, M. F. (1952).—*J. Gen. Chem. Moscow* **22** : 1457.
Metzenburg, R. L., and Mitchell, H. K. (1954).—*J. Amer. Chem. Soc.* **76** : 4187.

SYNTHESIS OF HELIOTRAMIDE

By R. Adams,* C. C. J. Culvenor,† C. N. Robinson,* and H. A. Stingl*

[*Manuscript received July 2, 1959*]

Summary

Both racemates of 4-methoxy-3-hydroxy-2-methylpentane-3-carboxamide are synthesized by reacting *iso*propylmagnesium chloride with 2-methoxypropionitrile, converting the resulting ketone into the cyanohydrin and hydrolysing to the amide. Comparison with heliotramide shows that the lower melting racemate, m.p. 81–82 °C, is (±)-heliotramide and thus of threo-configuration. The higher melting racemate, m.p. 145–146 °C, is the *erythro*-amide. Attempts to hydrolyse the amides to the carboxylic acids were unsuccessful.

Heliotramide is prepared by heating heliotric acid with urea or by the usual acid chloride-ammonia procedure following protection of the hydroxyl group.

I. Introduction

Two previous attempts to synthesize heliotric acid, 4-methoxy-3-hydroxy-2-methylpentane-3-carboxylic acid (I), have been reported. Adams and van Duuren (1953) obtained the structural isomer (II) by bromomethoxylation of 2-*iso*propylcrotonic acid with subsequent replacement of the halogen by hydroxyl. Employing methanolysis of the epoxide (III) as the key step, Dry and Warren (1955) prepared racemic *erythro*-4-methoxy-3-hydroxy-2-methylpentane-3-carboxylic acid which they considered was not the racemic form of heliotric acid. This conclusion is in agreement with the deduction that heliotric acid has the threo-configuration since it is demethylated by concentrated hydrobromic acid to trachelanthic acid (IV) which is known to have the threo-configuration (Adams and van Duuren 1952, 1953 ; Dry and Warren 1952).

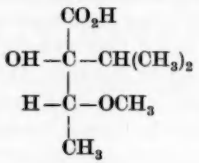
In the present attack on this problem a sterically non-specific synthesis has been employed to prepare both racemic forms of the corresponding amide, 2-*iso*propyl-2-hydroxy-3-methoxybutyramide (V). 2-Methoxy-4-methylpentan-3-one (VI), prepared from 2-methoxypropionitrile and *iso*propylmagnesium chloride, was converted into the cyanohydrin (VII) which was hydrolysed to the hydroxyamide (V). The resulting racemates, m.p. 81–82 °C and 145–146 °C, were readily separated by means of their different solubilities in light petroleum but all attempts to hydrolyse them to the desired carboxylic acids have been unsuccessful.

The racemic amides have been compared with the amide of heliotric acid and relative configurations assigned on this basis. Heliotramide, m.p. 65–66 °C, is readily obtained by fusing heliotric acid with urea. It was also prepared by way of the acid chloride after protection of the hydroxyl group by acetylation.

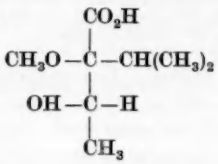
* University of Illinois, Urbana, Ill., U.S.A.

† Organic Chemistry Section, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

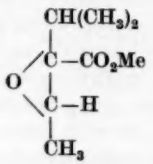
Although reaction of an ester with liquid ammonia at high temperatures has been recommended for preparation of the amides of hydroxy acids (Audrieth and Klimberg 1938) this method failed in the present instance. The infra-red spectra of heliotramide and the racemate of m.p. 81–82 °C in carbon tetrachloride and in



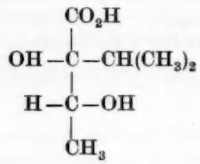
(I)



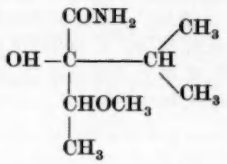
(II)



(III)



(IV)



(V)

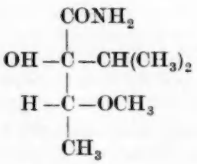
$$CH_3CH(OCH_3)Cl \xrightarrow{CuCN} CH_3CH(OCH_3)CN$$

$$\xrightarrow{iso\text{-}C_5H_{11}MgCl} CH_3CH(OCH_3)COCH(CH_3)_2$$

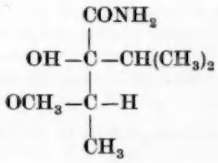
(VI)

$$\xrightarrow{HCN} CH_3CH(OCH_3)-\underset{\displaystyle CH(CH_3)_2}{\overset{\displaystyle OH}{\underset{|}{\overset{|}{C}}}}-CN \xrightarrow{H_2SO_4} \text{(V)}$$

(VII)



(VIII)

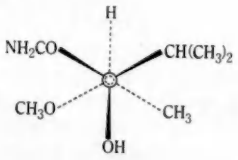


(IX)

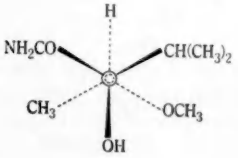
chloroform solutions are identical. This racemate therefore has the threo-configuration (VIII) and the racemate of m.p. 145–146 °C the erythro-configuration (IX).

These assignments of configuration are supported by the infra-red spectra in so far as these provide evidence for intramolecular bonding of NH in the racemate, m.p. 81–82 °C, and intermolecular bonding in that of m.p. 145–146 °C.

The *threo*-amide (VIII) would be expected to have the preferred conformation X in which the H-atom on C_4 is between the two large substituents on C_3; this conformation permits intramolecular hydrogen bonding between the NH_2 and OMe groups. The *erythro*-amide (IX), in the equivalent conformation XI, would be unable to form such a hydrogen bond involving a six-membered ring. Formation of the six-membered chelate ring in X tends to bring a hydrogen atom into eclipse with the *iso*propyl group whereas in XI it would tend to bring the larger methyl group into eclipse. Intramolecular bonding between $CONH_2$ and OH groups, or between OH and OMe groups, is possible for both forms but would involve formation of five-membered rings. Such hydrogen bonds are much weaker than bonds in six-membered rings. The solution spectra show an unbonded carbonyl group in both racemates; in Nujol, the CO group is unbonded in the racemate m.p. 81–82 °C and bonded in that of m.p. 145–146 °C. The relative melting points and solubilities of the racemates are in agreement with that of m.p. 81–82 °C (soluble in light petroleum), being intramolecularly bonded and that of m.p. 145–146 °C (almost insoluble in light petroleum), being intermolecularly bonded.



(X)



(XI)

In another attempt at synthesis of heliotric acid, 2-methoxy-5-methylhexan-3,4-dione (XII) was prepared by the following procedure:

$$CH_3.CH(CN).OCH_3 \xrightarrow{iso\text{-}C_4H_9MgCl} CH_3.CH(OCH_3).CO.CH_2.CH(CH_3)_2$$

$$\longrightarrow CH_3.CH(OCH_3).CO.\underset{\underset{NOH}{\|}}{C}.CH(CH_3)_2$$

$$\longrightarrow CH_3.CH(OCH_3).CO.CO.CH(CH_3)_2$$

(XII)

However rearrangement of XII to the desired hydroxy acid could not be achieved.

II. Experimental

(*a*) *2-Methoxypropionitrile.*—A solution of chloroethyl methyl ether (331 g) (Wallace and Henze 1942), b.p. 68–70 °C, in dry ether (1 l.) was added slowly through a dropping funnel to a rapidly stirred suspension of dry cuprous cyanide powder (340 g) in dry ether. After the vigorous reaction had subsided, the mixture was refluxed on a steam-bath for 3 hr. It was then decanted from the residual salts which were washed several times with ether. The ether was removed from the combined extracts and the residual oil distilled. The yield after two redistillations was 160 g (54%); b.p. 112·5–116·5 °C, n_D^{20} 1·3830. Wallace and Henze (1942) report a yield of 36%, but give no experimental details.

(*b*) *2-Methoxy-4-methylpentan-3-one.*—To the Grignard reagent, prepared from magnesium (13 g) and *iso*propyl chloride (42 g) in ether (300 ml), was added with vigorous stirring a solution of 2-methoxypropionitrile (40 g) in ether (120 ml). The reaction mixture was allowed to stand for 17 hr. Cautious addition of water (60 ml) was followed by N hydrochloric acid (300 ml) and enough 3N hydrochloric acid to obtain a clear solution in both layers. The aqueous layer was extracted three times with ether, and the combined ethereal solution dried over magnesium sulphate. After evaporation of the solvent the residue was distilled *in vacuo* to give 68 g of a product, b.p. 61–76 °C at 50 mm. Upon refractionation, 39 g (64%) of ketone resulted; b.p. 45·5–47·5 °C at 19 mm, n_D^{21} 1·4070 (Found: C, 64·0; H, 10·4%. Calc. for $C_7H_{14}O_2$: C, 64·6; H, 10·8%). Entirely satisfactory analytical results were not obtained; this coincided with the findings of previous investigators. Wallace and Henze (1942) report a yield of 13% of product, b.p. 58 °C at 31 mm, n_D^{20} 1·4092.

(*c*) *2-iso*Propyl-2-hydroxy-3-methoxybutyronitrile.*—A solution of 2-methoxy-4-methylpentane-3-one (13 g) in liquid hydrogen cyanide (5 g) was treated at 0 °C with one drop of a saturated aqueous solution of potassium cyanide, and the mixture was stoppered and allowed to stand at that temperature for 14 hr. The cyanohydrin was then stabilized with a drop of concentrated sulphuric acid and allowed to stand at room temperature for 10 hr. The remaining hydrogen cyanide was removed under reduced pressure and the residual yellow oil distilled to give 13·7 g (88%) of *2-isopropyl-2-hydroxy-3-methoxylbutyronitrile*, b.p. 61–62 °C at 0·5 mm (Found: C, 59·9; H, 9·5; N, 9·1%. Calc. for $C_8H_{15}O_2N$: C, 61·1; H, 9·6; N, 8·9%).

(*d*) *2-iso*Propyl-2-hydroxy-3-methoxybutyramide.*—A solution of concentrated sulphuric acid (20 ml) in water (2 ml) was cooled to 0 °C and added to *2-iso*propyl-2-hydroxy-3-methoxybutyronitrile (13 g). After standing at 0 °C for 3·5 hr and at room temperature for 16 hr, it was poured over cracked ice (c. 200 g). After the ice had melted, the solution was extracted thoroughly with chloroform. The yellow oil from the extracts solidified after standing for a few hours under vacuum. This solid (11 g, 76%) was a mixture of stereoisomers. It was transferred to a Soxhlet extractor and extracted with light petroleum (b.p. 30–60 °C) for 6 hr. The white residue weighed 0·9 g, m.p. 142–143 °C. The extract was evaporated to dryness and the solid residue extracted a second time for 45 min with light petroleum. The insoluble material (0·85 g) brought the total yield of high-melting, less-soluble amide to 1·75 g. This was extracted over a period of 40 hr with light petroleum and small, colourless crystals of the *erythro-form* (*XI*) separated, m.p. 145–146 °C (Found: C, 55·0; H, 9·5; N, 8·1%. Calc. for $C_8H_{17}O_3N$: C, 54·8; H, 9·8; N, 8·0%).

The light petroleum from the second extraction above was placed in the refrigerator overnight, whereupon pure, *threo-form* (*X*) (8·25 g) separated in large prisms, m.p. 79–81 °C. Chromatography on alumina and recrystallization from light petroleum raised the melting point to 81–82 °C (Found: C, 55·1; H, 9·6; N, 8·2. Calc. for $C_8H_{17}O_3N$: C, 54·8; H, 9·8; N, 8·0%).

(*e*) *Attempted Hydrolysis of the Low-Melting Racemic 2-iso*Propyl-2-hydroxy-3-methoxybutyramide.*—A variety of methods were studied for hydrolysis of the amide; concentrated sodium hydroxide, glycolic and ethanolic potassium hydroxide, ethanolic sulphuric acid and sodium nitrite, 80% sulphuric acid, 100% phosphoric acid, hydrogen chloride and amyl nitrite in benzene or dry dioxane, concentrated sulphuric acid, and saturated aqueous sodium nitrite. All were unsuccessful.

(*f*) *Heliotramide.*—(i) Heliotric acid (10 g) and urea (20 g) were heated at 150–160 °C for 6 hr. A homogeneous liquid formed when the urea melted and a sublimate appeared on the upper walls of the tube. The reaction mixture was cooled, dissolved in water (100 ml), made alkaline to phenolphthalein, and extracted with ether. The resulting gum (1·1 g) was taken up in benzene, filtered, and re-evaporated. The residue was dissolved in a fairly small volume of light petroleum (b.p. <40 °C), decanted from insoluble material, and cooled in a refrigerator. Seeding with crystals which were produced by leaving some of the original gum in a desiccator for several days, gave colourless prisms or tablets of *heliotramide*, m.p. 63–64 °C. Recrystallization gave m.p. 65–66 °C (Found: C, 54·8; H, 9·6; N, 8·1. Calc. for $C_8H_{17}O_3N$: C, 54·8; H, 9·8; N, 8·0%). Specific rotation, $[\alpha]_D^{21}$ +11·8° (*c*, 2·2 in ethanol). The amide is readily soluble in water and in all

organic solvents, including light petroleum, at room temperature. Because of its high solubility, it is possible that small yields of amide would have been missed in earlier attempts at preparation. In a second run, heliotric acid (8 g) was heated with urea (24 g) for 60 hr at 145–160 °C. Working up as before gave crude amide (2·8 g) and recovered heliotric acid (4·7 g).

(ii) Heliotric acid (1 g) was refluxed in pyridine (10 ml) with acetic anhydride (1·3 ml) for 3 hr. The mixture was poured into dilute sulphuric acid and extracted with light petroleum to give a gum (0·50 g) which crystallized after several hours. Extraction of the aqueous residue with ether gave additional product (0·30 g) which also crystallized. Both products had R_F 0·82 in butanol–acetic acid and R_F 0·55 in butanol–ammonia (cf. heliotric acid, R_F 0·80 in butanol–acetic acid, R_F 0·49 in butanol–ammonia). No suitable method was found for recrystallization of acetylheliotric acid and it was used in the crude state. The acetylated acid (0·5 g) was dissolved in benzene (2 ml), treated with thionyl chloride (3 ml), and kept for 3 hr at room temperature. The mixture was evaporated under reduced pressure and any trace of thionyl chloride removed by addition of more benzene and re-evaporation. The residue was taken up in dry ether (2 ml) and shaken with conc. aqueous NH_3 (25 ml) for 1·5 hr. Dilution with water and extraction with ether gave a light amber gum (0·33 g) which was heated in 1N NaOH (15 ml) for 1·5 hr at 100 °C. Extraction of this solution with ether gave a gum (0·11 g) which was readily soluble in light petroleum (b.p. <40 °C). Cooling this solution to 0 °C and seeding with heliotric acid amide gave prisms (106 mg), m.p. 63–65 °C, mixed m.p. 64–65 °C.

(*g*) *2-Methoxy-5-methylhexan-3-one.*—This was prepared by the method used for 2-methoxy-4-methylpentan-3-one. From magnesium (13·1 g), *iso*butyl chloride (50 g) in ether (300 ml), and 2-methoxypropionitrile (42·5 g) was obtained ketone, after redistillation (44 g, 61%), b.p. 59–61·5 °C at 15 mm. Wallace and Henze (1942) report a yield of 21% of product, b.p. 51–52 °C at 9 mm, by the use of *iso*butyl bromide for the Grignard reagent.

(*h*) *2-Methoxy-4-isonitroso-5-methylhexan-3-one.*—Into a stirred solution of ketone (19·5 g) in dry ether (100 ml), methyl nitrite (*Org. Synth. Coll.* **2**: 363) and dry hydrogen chloride were introduced simultaneously. The hydrogen chloride must be introduced very slowly (less than two bubbles per sec) to avoid cleavage of the ketone molecule.

The solution developed a brown-red and later a greenish colour but became brown again upon standing overnight. Nitrogen was then introduced for 15 min to remove excess hydrogen chloride and the reaction mixture was extracted with four 20-ml portions of 10% aqueous sodium hydroxide. The alkaline extracts were combined, washed with ether, and acidified with cooling with concentrated hydrochloric acid (*c.* 14 ml). By thorough extraction of the acidified solution with ether, drying, removal of solvent, and fractionation of the residue, *isonitrosoketone* (5·5 g) (23·6%) was obtained, b.p. 88–92 °C at 0·3 mm, n_D^{20} 1·4590 (Found: N, 7·9%. Calc. for $C_8H_{15}O_3N$: N, 8·1%). Starting ketone (about 4·2 g) could be recovered by distillation of the ether washings.

Sometimes an oil separated from the reaction mixture. This was either dissolved by adding more ether or separately dissolved in alkali and combined with the other alkaline extracts. If the hydrogen chloride was introduced too rapidly, hydroxylammonium chloride precipitated from the reaction solution. The alkali-soluble material also contained a lower-boiling fraction consisting of *iso*valeric and propionic acids, identified as their *p*-phenylphenacyl esters.

(*i*) *2-Methoxy-5-methylhexan-3,4-dione.*—A mixture of the above *iso*nitrosoketone (3·5 g) and 3N H_2SO_4 (50 ml) was refluxed gently for 20 min, then steam distilled. The yellow upper layer of the distillate was collected, the aqueous layer was four times extracted with ether, the ether extracts were combined with the yellow oil, dried over anhydrous sodium sulphate, and distilled to give the *diketone* (1·8 g, 57%), b.p. 66–70 °C at 14 mm, n_D^{20} 1·4150 (Found: C, 60·4; H, 9·0%. Calc. for $C_8H_{14}O_3$: C, 60·7; H, 8·9%). Attempts to rearrange the diketone to an hydroxy acid were unsuccessful. With 2,4-dinitrophenylhydrazine a *monohydrazone* was formed; light orange crystals from ethanol, m.p. 143–144 °C (Found: N, 16·5%. Calc. for $C_{14}H_{18}O_6N_4$: N, 16·7%).

From equimolar amounts of diketone and *o*-phenylenediamine, heated in glacial acetic acid for 2 hr to 95 °C, *2-(1′-methoxyethyl)-3-isopropylquinoxaline* precipitated upon pouring onto ice. It formed colourless crystals from dilute ethanol, m.p. 60·5–61 °C (Found: C, 73·1; H, 7·8%. Calc. for $C_{14}H_{18}ON_2$: C, 73·0; H, 7·9%).

(*k*) *Infra-Red Absorption Spectra.*—The relevant absorption bands shown by heliotramide and the synthetic racemates are listed in Table 1. The peaks quoted for solutions are for 2·5% concentration, cell thickness 0·2 mm. More concentrated solutions, 10% (0·05 mm cell) for the racemate, m.p. 81–82 °C, and 5% (0·05 mm cell) for the racemate, m.p. 145–146 °C, were also measured but the peak positions were unaltered. Intensity differences between solution and mull were very slight for the racemate, m.p. 81–82 °C, and small but appreciable for certain bands for the racemate m.p. 145–146 °C.

TABLE 1

INFRA-RED ABSORPTION PEAKS

sh, shoulder ; br, broad ; w, weak ; s, strong ; m, medium

Compound, Region	CCl_4 Solution (cm^{-1})	$CHCl_3$ Solution (cm^{-1})	Nujol Mull (cm^{-1})
Heliotramide			
δ-NH_2			1615 (m)
Carbonyl	As for racemate m.p. 81–82 °C		1683 (s)
OH, NH stretching			3220 (s), 3300 (w) 3400 (s)
Racemate, m.p. 81–82 °C			
δ-NH_2	1560 (w), 1585 (s) 1600 (sh)	1560 (w), 1585 (s)	1620 (m)
Carbonyl	1688 (s)	1682 (s)	1683 (s)
OH, NH stretching	3200 (br), 3300 (w) 3400 (sh), 3450 (s) 3500 (sh)	3200 (br), 3380 (m) 3500 (s)	3190 (s), 3320 (sh) 3380 (s)
Racemate, m.p. 145–146 °C			
δ-NH_2	*	1565 (s)	1600 (br)
Carbonyl	*	1683 (s)	1657 (s)
OH, NH stretching	*	3330 (sh), 3410 (s) 3490 (sh), 3540 (s) 3590 (sh)	3150 (br), 3300 (s) 3420 (s)

* Not measured, because of sparing solubility.

III. ACKNOWLEDGMENTS

The authors from the University of Illinois are grateful to the A. P. Sloan Foundation for financial support which made possible their part of the investigation. Dr. J. B. Willis and Dr. C. S. Barnes are thanked for helpful discussion.

IV. REFERENCES

ADAMS, R., and VAN DUUREN, B. L. (1952).—*J. Amer. Chem. Soc.* **74** : 5349.
ADAMS, R., and VAN DUUREN, B. L. (1953).—*J. Amer. Chem. Soc.* **75** : 4636.
AUDRIETH, C. F., and KLIMBERG, J. (1938).—*J. Org. Chem.* **3** : 312.
DRY, L. J., and WARREN, F. L. (1952).—*J. Chem. Soc.* **1952** : 3445.
DRY, L. J., and WARREN, F. L. (1955).—*J. Chem. Soc.* **1955** : 65.
WALLACE, W. P., and HENZE, H. R. (1942).—*J. Amer. Chem. Soc.* **64** : 2882.

STUDIES OF CASEIN

I. SOME OBSERVATIONS ON THE HETEROGENEITY OF CASEIN FRACTIONS

By H. A. McKenzie* and R. G. Wake†

[*Manuscript received January* 30, 1959]

Summary

The heterogeneity of casein is discussed in the light of methods currently used for the fractionation of casein. In particular, the possible heterogeneity of certain preparations of α-casein is considered. This is important because it has been generally considered that α-casein is the protective colloid which is altered when the enzyme, rennin, acts on casein micelles. Recently, Waugh and von Hippel (1956) have suggested that their new component κ-casein, and not α-casein, is the protective colloid. These two viewpoints could be reconciled if α-casein samples previously examined contained κ-casein as well. In the present work, a study is made of filter paper electrophoresis, micelle-forming properties, and sedimentation of casein fractions. It is shown that κ-casein is concentrated with α-casein in fraction A during the alcohol fractionation method of Hipp *et al.* (1952). On the other hand fraction B contains α-casein essentially free of κ-casein. The α-casein obtained in the urea fractionation method of Hipp *et al.* also contains κ-casein. Thus only alcohol fraction B is a suitable source of pure α-casein.

During the paper electrophoretic examination of casein fractions a number of minor protein components are observed. A component moving more slowly than γ-casein is present in acid casein, second-cycle casein—fraction P, and an alcohol fraction. This component was first observed in the latter fraction by Hipp *et al.* (1952) when preparing γ-casein. Electropherograms of second-cycle casein—fraction S indicate the presence of κ-, β-, and γ-casein, and two minor components moving between κ- and β-. The way in which these components arise is briefly discussed.

I. Introduction

Despite considerable investigation many aspects of the chemistry and biochemistry of casein remain obscure. In the present series of papers the authors will be concerned with throwing new light on problems such as the heterogeneity of casein, the molecular size of the caseins, and the mode of action of the milk clotting enzyme, rennin, on casein micelles.

The fact that the composition and properties of preparations of acid casein (Hammarsten 1883) were surprisingly constant led to the idea that casein was a pure protein. However, in 1925 Linderstrøm-Lang and Kodama (1925) showed

* Division of Food Preservation and Transport, C.S.I.R.O., Physico-Chemical Unit, Biochemistry Department, University of Sydney; present address: Department of Physical Biochemistry, Australian National University, Canberra, A.C.T.

† Biochemistry Department, University of Sydney; present address: Department of Biochemistry, Stanford University, California.

by solubility studies that casein is heterogeneous. This work led to various attempts to separate the casein components (Linderstrøm-Lang 1929; Cherbuliez and Meyer 1933; Groh *et al.* 1934). Mellander (1939) found that, on moving

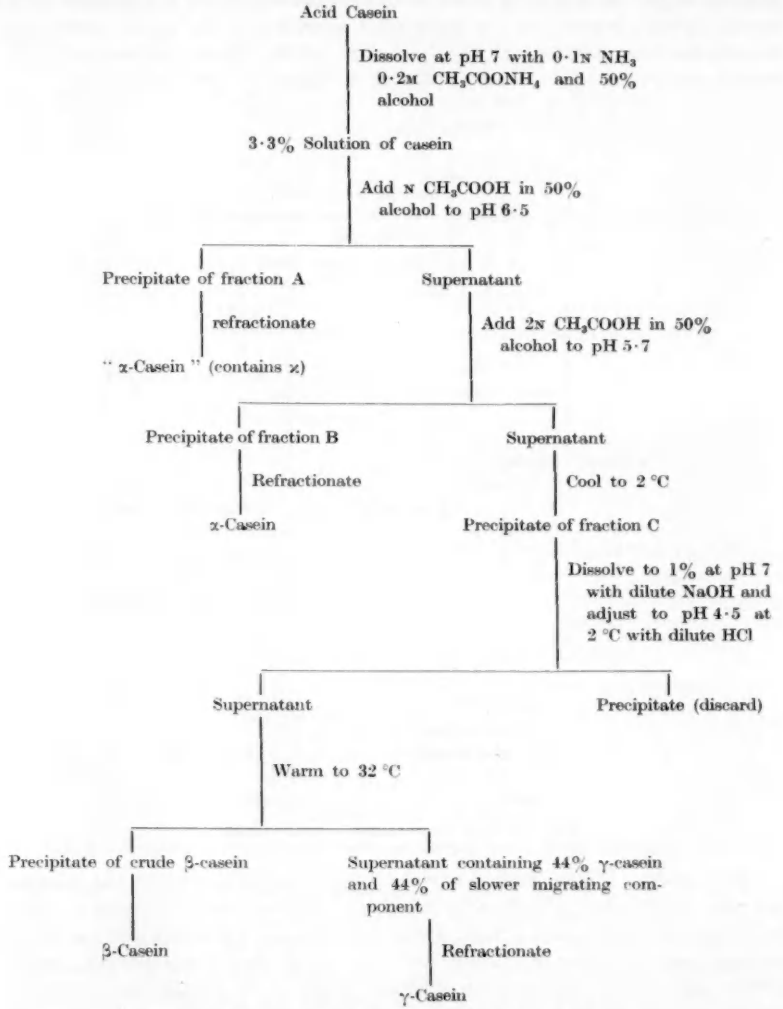


Fig. 1.—Alcohol fractionation of acid casein.

boundary electrophoresis, casein showed three components, which he designated α-, β-, and γ- in decreasing order of mobility. Warner (1944) developed a method for separating α- and β-casein based on the higher solubility of β- at

pH 4·4 and 2 °C. Subsequently, Hipp *et al.* (1952) devised two methods for the fractionation of casein into three components. The first procedure is based on differences in solubility of α-, β-, and γ-casein in 50 per cent. alcohol in the presence of salt, as well as in water, with changes in pH and temperature. The second method depends on the differential solubility of the casein components in aqueous urea solutions at the isoelectric point. These methods (modified slightly by us) are schematically outlined in Figures 1 and 2.

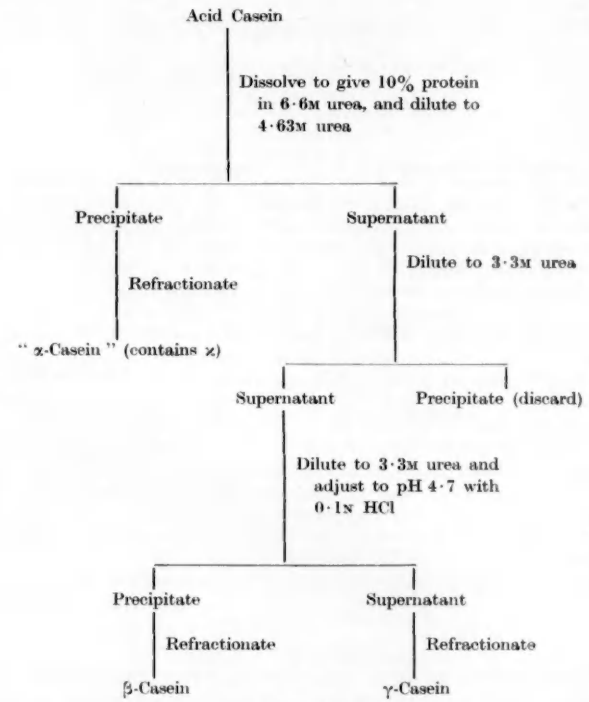


Fig. 2.—Urea fractionation of acid casein.

The casein in skim milk occurs in various colloidal states: as micelles (the size distribution of which is relatively narrow—see Nitschmann (1949) and Hostettler and Imhof (1951*a*, 1951*b*)), and small aggregates and monomers (i.e. single casein molecules). These forms are presumably in equilibrium, and in addition the casein incorporates calcium, which is in equilibrium with free calcium(II) and calcium phosphate. It is surprising, therefore, that little use has been made of these equilibria to fractionate casein. An important contribution has recently been made in this direction by Waugh and his colleagues (von Hippel and Waugh 1955; Waugh and von Hippel 1956). They separated the casein from whey protein by centrifugation after adding calcium(II) (to

0·06M) at pH 7. This first-cycle casein* was made 0·25M in calcium(II) at 2 °C. Two fractions were obtained as outlined in Figure 3. Second-cycle casein—fraction P was considered primarily a mixture of α- and β-casein and second-cycle casein—fraction S a mixture of β-casein and a new component called ϰ-casein.

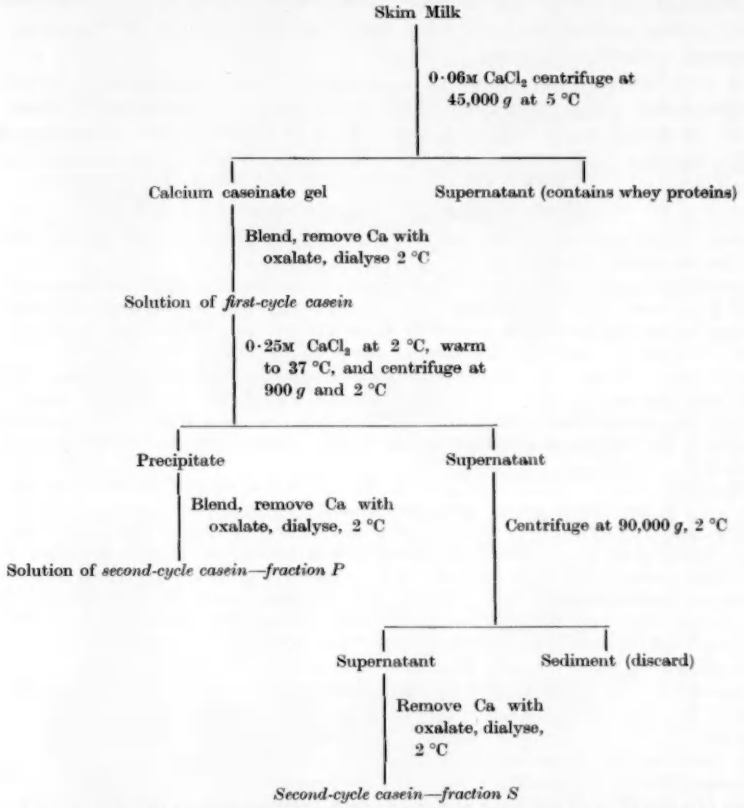


Fig. 3.—Preparation of first-cycle casein and second-cycle casein—fractions P and S.

The latter was not isolated in a pure state. Waugh and von Hippel claim that ϰ-casein is present in skim milk as an α-ϰ complex, which is split during the second cycle of their preparation, the ϰ-casein being concentrated in fraction S. They state that " ϰ-casein is the most important single factor responsible for micelle stabilization and it is the protein on which rennin acts immediately ".

* von Hippel and Waugh referred to their casein as *soluble* casein. The present authors consider that the term " soluble casein " is undesirable (e.g. acid casein can be made which is readily soluble at neutral pH). First- and second-cycle soluble casein and fraction S will be referred to as first-cycle casein, second-cycle casein—fraction P, and second-cycle casein—fraction S respectively.

Earlier Linderstrøm-Lang (1929) suggested that the activity of rennin is due to its ability to destroy or inactivate a " protective colloid " component whose function is to keep the other calcium caseinates in " solution ". Most of the evidence prior to the work of von Hippel and Waugh in 1956 has pointed to α-casein as the " protective colloid ". The two viewpoints may be reconciled if the earlier conclusions have been based on preparations of " α-casein " containing κ-casein.

It is obviously of importance to isolate pure α- and κ-casein and determine their properties. As a preliminary to this, it is necessary to examine the heterogeneity of various casein fractions, especially with regard to the occurrence of the α-κ complex, and α- and κ-casein. Such observations are reported in the present paper.

II. Materials and Methods

Milk : In all fractionation procedures fresh, unpasteurized pooled samples of whole milk were used as starting material.

Total Milk Protein : Skim milk (1 l.) was treated with 200 ml 1·5M $K_2C_2O_4$ at constant pH (cf. von Hippel and Waugh 1955), filtered through Whatman No. 3 paper, centrifuged at 90,000 *g* for 60 min (Spinco Preparative Ultracentrifuge Model L, rotor 30 ; 28,000 r.p.m.), and dialysed exhaustively against 0·1M NaCl. The whole procedure was carried out at 2 °C.

Acid Casein : This was prepared at room temperature by the method of Hipp *et al.* (1952) except, after the first dissolution, the opalescent solution was clarified by filtration through a Whatman No. 1 paper before reprecipitation. This gave a final product which was completely soluble as a clear solution at pH 7·0, providing care was taken in the acid precipitation and dissolution.

Alcohol Fractionation of Casein : Acid casein was dissolved in water at pH 7·0 by the slow addition of 0·1N ammonia to give an approx. 6% solution. This was fractionated by the alcohol method of Hipp *et al.* (1952) to give the three preliminary fractions A, B, and C (see Fig. 1). Each of these fractions, as well as a sample of acid casein, was dissolved in dilute NaOH to give an approx. 3% solution and then adjusted to pH 12·0. After standing for 3 hr at 2 °C, the solutions were dialysed against phosphate buffer to pH 7·0 and then against water to remove most of the salts. At this stage fractions B and C were clear while the others were slightly opalescent. The pH values of all solutions were between 6·5 and 6·7.

Waugh and von Hippel Caseins : First-cycle casein, second-cycle casein—fractions P and S were prepared according to Waugh and von Hippel (1956) except for the use of NaCl throughout instead of KCl (see Fig. 3).

α- and β-Casein : These were prepared from acid casein by the alcohol method of Hipp *et al.* (1952). Only the crude alcohol fraction B was used as a source of α-casein.

Storage of Protein Fractions : Acid casein, α-, and β-casein were dissolved at pH 7·0 by the addition of N NaOH. The solutions were dialysed exhaustively against water at 2 °C. These and other solutions of protein fractions were freeze-dried and stored at 2 °C.

Protein Concentrations : These were obtained by drying samples of stock protein solutions to constant weight at 105 °C.

Reagents and Buffers : A.R. chemicals were used except where otherwise stated. The phosphate buffer, pH 7·0, *I* 0·1, was prepared by dissolving 1·18 g $NaH_2PO_4.2H_2O$, 2·02 g Na_2HPO_4, and 2·92 g NaCl in 1 l. water. The veronal buffer at pH 8·3, *I* 0·1, was prepared by diluting 40 ml 0·5M sodium veronal (May and Baker, B.P. Grade), 2·65 ml 2N HCl, and 16 ml 5M NaCl to 1 l. Measurements of pH were made at room temperature with a type 1199–44 Leeds and Northrup glass electrode using a Leeds and Northrup type 7666 pH indicator. For pH values > 10, a Doran type M 5969 alkacid glass electrode was used. Values of standard buffer solutions and general procedure were in accordance with the recommendations of Bates (1954). The meaning to be attached to the pH measurements in alcohol–water mixtures is also discussed by Bates (1954).

STUDIES OF CASEIN. I

Fig. 1.—Electrophoretic patterns obtained for (*a*) total milk protein ; (*b*) first-cycle casein ; (*c*) second-cycle protein—fraction P; (*d*) second-cycle protein—fraction S. The run was carried out in phosphate at pH 7·0, *I* 0·1, 2°C on Whatman No. 1 paper for 20 hr at 200 V.

Fig. 2.—Electrophoretic patterns obtained for (*a*) acid casein ; (*b*) fraction A ; (*c*) fraction B ; (*d*) fraction C ; conditions as in Figure 1.

STUDIES OF CASEIN. I

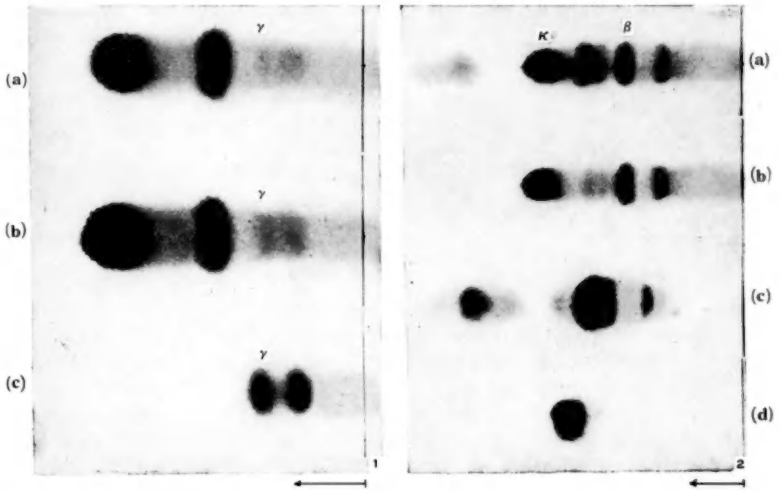


Fig. 1.—Paper electrophoretic patterns for (*a*) acid casein; (*b*) second-cycle casein—fraction P; (*c*) alcogol fraction containing component moving more slowly than γ-casein. The run was carried out in veronal at pH 8·3, *I* 0·1, and 2 °C on Schleicher and Schull No. 2043B paper for 16 hr at 170 V.

Fig. 2.—Paper electrophoretic patterns for (*a*) second-cycle casein—fraction S; (*b*) precipitate; (*c*) supernatant obtained by exhaustive dialysis of fraction S; (*d*) β-lactoglobulin; conditions as in text Figure 4.

Micelle Forming Properties : These were investigated using the rapid mixing technique of Waugh and von Hippel (1956).

Moving Boundary Electrophoresis and Ultracentrifugation : See Parts II and III of this series for details.*

Paper Electrophoresis : These measurements were made at 2 °C using an LKB type 3276 paper electrophoresis apparatus. In all cases approx. 0·01 ml 5% protein solution was applied as a spot to the paper. The protein was dyed with bromphenol blue (Kunkel and Tiselius 1951) after drying at 110 °C for 30 min.

III. Results

(*a*) *Filter Paper Electrophoresis*

Electropherograms for total skim milk protein and casein fractions, prepared according to the method of Waugh and von Hippel, in phosphate buffer of pH 7·0 and *I* 0·1, are shown in Plate 1, Figure 1. It will be noted that the leading component (designated α-κ) in both total milk protein and first-cycle casein moves at an intermediate rate between that of the leading α component in second-cycle casein—fraction P and that of the κ component in second-cycle casein—fraction S. A similar distribution of mobilities is observed on moving boundary electrophoresis (see Part II of this series). These observations are in accord with the contention that there is an α-κ complex in skim milk and first-cycle casein and that this complex is broken during the second cycle.

Results for acid casein and the alcohol fractions A, B, and C in the pH 7·0 phosphate buffer are shown in Plate 1, Figure 2. It will be noted that the faster moving component in acid casein and the leading component in fraction A move at approximately the same speed. Both move more slowly than the leading component in fraction B. The results suggest that the κ-casein is concentrated in fraction A along with α-casein. Fraction B contains mainly, if not entirely, α-casein as the fast moving component, while β-casein is concentrated in fraction C.

In measurements both with filter paper and the moving boundary method the distance moved by the β-component depends on the distance moved by the leading component. This effect is due to interactions between the β-casein and other components. The effect could be reproduced with synthetic mixtures of pure α- and β-, and κ- and β-casein (for the preparation of κ- see Part IV of this series (Wake 1959)). The β-casein had no effect on the rate of migration of α- and κ-casein. However, α-casein greatly enhanced the rate of movement of β-casein, while κ-casein produced no noticeable effect. When all three components were mixed the formation of the α-κ complex, moving at an intermediate rate between those of the separate components, was clearly demonstrated. The effect of the α-κ complex on the rate of movement of β- was less than that of α- alone.

During the paper electrophoretic examination of various casein fractions a number of minor protein components were observed. Invariably present to approximately the same extent as γ-casein in preparations of acid casein and various casein fractions was a component moving more slowly than γ-casein. The only other similar component which appears to have been noted by previous

* See following papers this journal, pp. 723, 734.

workers is that observed by Hipp *et al.* (1952) in an alcohol fraction used for preparing γ-casein. Therefore a comparison was made of electropherograms for acid casein, second-cycle casein—fraction P, and an alcohol fraction similar to that of Hipp *et al.* These results are shown in Plate 2, Figure 1, and the similarity is obvious. Moving boundary electrophoretic patterns of the alcohol fraction are shown in Figure 4. The γ component had a mobility of $-2{\cdot}02\times10^{-5}$ cm^2 V^{-1} sec^{-1} in agreement with other workers. The slow moving component had a mobility of $-0{\cdot}94\times10^{-5}$ cm^2 V^{-1} sec^{-1}.

Electropherograms of second-cycle casein—fraction S indicated the presence of κ-, β-, and γ-casein. In addition there were two minor components moving between κ- and β-casein. Occasionally it was possible to detect the minor component which moves more slowly than γ-casein. Waugh and von Hippel

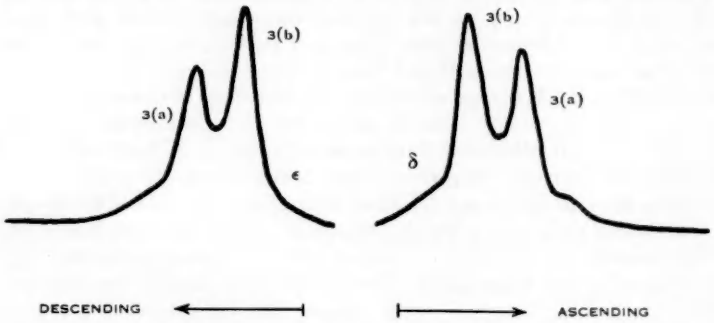


Fig. 4.—Moving boundary electrophoresis of alcohol fraction of casein containing component moving more slowly than γ-casein ; 3 (*a*) γ-casein, 3 (*b*) additional component. The run was carried out with 0·5 per cent. protein in veronal at pH 8·3, *I* 0·1, and 4·12 V/cm ; 178 min, θ, 20°.

(1956) only reported the presence of κ- and β-casein in fraction S. Exhaustive dialysis of fraction S against water at 2 °C caused precipitation of most of the protein as the pH fell from 7·0 to 5·2. Electropherograms of this precipitate and the supernatant (after freeze-drying), together with those of fraction S and β-lactoglobulin, are shown in Plate 2, Figure 2. The two minor components migrating between κ- and β- are seen to be concentrated in the supernatant and to move more slowly than β-lactoglobulin under the same conditions. They are also concentrated in a single fraction during the large-scale preparation of κ-casein (see Part IV of this series), approximately 4 g being obtained from 18 l. of raw milk. It is unlikely that they are identical with any of the known whey proteins. Larson and Gillespie (1957) observed an " unidentified component with an electrophoretic mobility midway between α- and β-casein " during the urea fractionation of acid casein.

(*b*) *Sedimentation*

Sedimentation patterns obtained for second-cycle casein—fractions P and S, and fractions A and B in phosphate at pH 7·0, *I* 0·1, are shown in Figure 5. s_{20} values are indicated in Table 1.

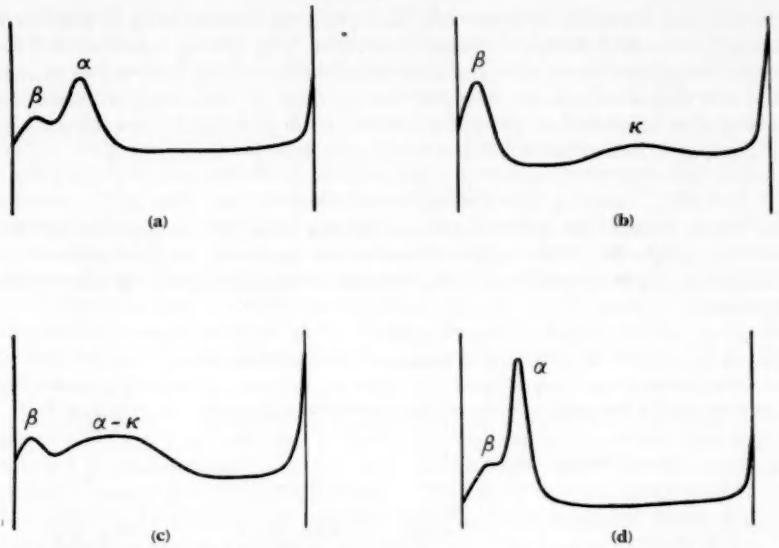


Fig. 5.—Sedimentation of casein fractions in phosphate at pH 7·0, I 0·1, and 2–5°C. Sedimentation is from left to right.
(*a*) Approx. 1·0 per cent. second-cycle casein—fraction P, 67 min, θ 70°; (*b*) approx. 1·0 per cent. second-cycle casein—fraction S, 76 min, θ 65 °C.; (*c*) approx. 1·5 per cent. fraction A, 71 min, θ 60°; (*d*) approx. 1·5 per cent. fraction B, 68 min, θ 70°.

The results obtained for fractions P and S are in general agreement with those described by Waugh and von Hippel (1956). The minor, slow moving component in fractions A and B represents β-casein. The presence of a diffuse and very fast-moving component in fraction A with s_{20} approximately 14 indicates

TABLE 1

APPROXIMATE S_{20} VALUES FOR CASEIN FRACTIONS (1 PER CENT. PROTEIN) IN PHOSPHATE, pH 7·0, I 0·1, 2–5 °C

The values given in brackets are those obtained by Waugh and von Hippel (1956) under similar conditions

Protein	Peak*				
	I	II	III	IV	V
First-cycle casein ..	(1·3)		(7·5)		
Second-cycle casein—P	1·4 (1·3)	4·2 (4·5)			
Second-cycle casein—S	1·3 (1·3)			10 (13·5)	
Fraction A	1·2				14
Fraction B	1·6	3·4			

* The compositions of the peaks are largely: I, β-casein; II, α-casein; III, α-κ complex; IV, κ-casein; V, κ- plus α-casein.

the presence of excess κ-casein. The faster and well-defined peak in fraction B has $s_{20}=3\cdot4$ and evidently represents α-casein. The low s_{20} value (cf. 4·2 for α-peak in fraction P) could be due to an effect similar to that observed by Waugh and von Hippel for the α-κ complex, the s_{20} value of which dropped from 7·5 to 6·5 after treatment at pH 12. Certainly there is no faster moving material in fraction B indicative of the α-κ complex or free κ-casein.

(c) *Micelle-Forming Properties*

Table 2 shows the results of an examination of various fractions for micelle-forming properties. The degree of cloudiness produced in the presence of calcium(II) has been taken to indicate the micelle-forming capacity of a particular fraction.

TABLE 2

MICELLE-FORMING PROPERTIES OF CASEIN FRACTIONS

All protein solutions used were 1·0 per cent. at pH 7·3; each tube contained 0·12 ml 4M NaCl and the volume was made up to 3·1 ml with water before adding 0·15 ml M $CaCl_2$ at 37 °C

Tube	Casein Fraction Solution (ml)					Observations			
						After 30 min at 37 °C		After 12 hr at 2 °C	
	Acid Casein	A	B	C	Second-Cycle Casein—Fraction S	Cloudiness	Ppt.	Cloudiness	Ppt.
1	2·0					****	o	****	o
2		2·0				**	o	**	o
3			2·0			o	****	o	***
4				2·0		Slight	****	o	o
5			2·0		1·0	*****	*	***	**
6					1·0	Slight	o	Slight	Slight

The absence of a precipitate and decreased cloudiness in tube 2 indicate the presence of excess κ-casein in fraction A, while the complete precipitation in tube 3 supports the absence of κ-casein from fraction B. Tube 5 shows that micelles can be formed with fraction B in the presence of added κ-casein. The behaviour of fraction C, tube 4, at 37 and 2 °C is consistent with the large proportion of β-casein. It is well known that calcium β-caseinate forms a colloidal suspension at room temperature, but is completely soluble at 2 °C.

α-Casein, isolated from fraction B, was completely precipitated by 0·05M $CaCl_2$, but gave stable micelles when mixed with fraction S prior to the addition of $CaCl_2$.

IV. GENERAL DISCUSSION

The foregoing work is in accord with the contention of Waugh and von Hippel that the new component, κ-casein, occurs normally in milk micelles as an α-κ complex. It is obvious that preparations of α-casein from certain fractions are mixtures of α- and κ-casein. Those workers who have obtained α-casein from

the alcohol fraction A of Hipp *et al.* (1952) have in fact prepared α-casein contaminated with κ-casein. The present work also indicates that pure α-casein can be prepared from the alcohol fraction B. Pure α-casein prepared from fraction B has been used in the study of this protein in Parts II, III, and V of this series.* The behaviour of " α-casein " prepared by Cherbuliez and Baudet (1950) using a method similar to Warner (1944), particularly its relative " solubility " in the presence of calcium(II), indicates that this material contains κ-casein. The behaviour, on rennin treatment, of " α-casein " obtained by Nitschmann and Keller (1955), using the urea method of Hipp *et al.* (1952), shows that this fraction is also contaminated with κ-casein (see Part V of this series). In the urea fractionation of casein the first dilution from 6·6 to 4·63M urea causes nearly complete precipitation of the α-κ complex. The small amount of β-casein is removed by further treatment under similar conditions so that what is finally obtained as " α-casein " is mainly a mixture of α- and κ-casein.

A method for the isolation of essentially pure κ-casein from second-cycle casein—fraction S is described in Part IV. Because of the strong interaction between β- and κ-casein, it is extremely difficult to remove the last traces of β-casein from preparations of the latter. This results in a low yield of κ-casein. An improved method for the preparation of κ-casein from acid casein will be described in a subsequent paper.

It is not known whether the three additional, minor components observed in the present work can be classed as integral parts of the casein complex. Possibly they are adsorbed onto the surface of the casein micelles and separated along with them when casein is prepared. They are possibly related to some of the microsomal proteins (Bailie and Morton 1958).

V. Acknowledgments

Grateful acknowledgment is made to Professor J. L. Still and Dr. J. R. Vickery in whose laboratories this work was carried out. Financial assistance and encouragement were received in the course of this work from Mr. G. Loftus Hills, Officer-in-Charge of the Dairy Research Section, C.S.I.R.O. Mr. M. B. Smith and Dr. F. E. Huelin have given considerable help during the work.

VI. References

Bailie, M. J., and Morton, R. K. (1958).—*Biochem. J.* **69**: 35.
Bates, R. (1954).—" Electrometric pH Determinations." (John Wiley & Sons: New York.)
Cherbuliez, E., and Baudet, P. (1950).—*Helv. Chim. Acta* **33**: 398.
Cherbuliez, E., and Meyer, F. (1933).—*Helv. Chim. Acta* **16**: 600.
Groh, J., Kardos, E., Denes, K., and Serenyi, Z. (1934).—*Z. physiol. Chem.* **226**: 32.
Hammarsten, O. (1883).—*Z. physiol. Chem.* **7**: 227.
Hipp, N. J., Groves, M. L., Custer, J. H., and McMeekin, T. L. (1952).—*J. Dairy Sci.* **35**: 272.
von Hippel, P. H., and Waugh, D. F. (1955).—*J. Amer. Chem. Soc.* **77**: 4311.
Hostettler, H., and Imhof, K. (1951*a*).—*Milchwissenschaft* **6**:, 351.
Hostettler, H., and Imhof, K. (1951*b*).—*Milchwissenschaft* **6**: 400.

* For Part V see *Aust. J. Biol. Sci.* **12**: 538.

KUNKEL, H. G., and TISELIUS, A. (1951).—*J. gen. Physiol.* **35**: 59.
LARSON, B. L., and GILLESPIE, D. C. (1957).—*J. Biol. Chem.* **227**: 565.
LINDERSTRØM-LANG, K. (1929).—*C.R. Lab. Carlsberg* (ser. chim.) **17** (9): 1.
LINDERSTRØM-LANG, K., and KODAMA, S. (1928).—*C.R. Lab. Carlsberg* (ser. chim.) **16** (1): 1.
MELLANDER, O. (1939).—*Biochem. Z.* **300**: 240.
NITSCHMANN, H. (1949).—*Helv. Chim. Acta* **32**: 1258.
NITSCHMANN, H., and KELLER, N. (1955).—*Helv. Chim. Acta* **38**: 942.
WAKE, R. G. (1959).—*Aust. J. Biol. Sci.* **12**: 538.
WARNER, R. C. (1944).—*J. Amer. Chem. Soc.* **66**: 1725.
WAUGH, D. F., and VON HIPPEL, P. H. (1956).—*J. Amer. Chem. Soc.* **78**: 4576.

STUDIES OF CASEIN

II. MOVING BOUNDARY ELECTROPHORESIS OF CASEIN FRACTIONS WITH PARTICULAR REFERENCE TO α-CASEIN

By H. A. McKenzie* and R. G. Wake†

[*Manuscript received January* 30, 1959]

Summary

A moving boundary electrophoretic study is made of acid casein, total milk protein, first-cycle casein, second-cycle casein—fractions P and S, α-casein (free of κ-casein), and β-casein.

Two acid-casein preparations are examined over the pH range 6·3 to 8·8 (*I* 0·02 and 0·1). The two preparations behave slightly differently from each other, but both show differences from that examined by Warner (1944). In veronal buffer, pH 8, the splitting of the " α peak " is observed only after prolonged electrophoresis. However, it occurs even at 1 per cent. concentration and in phosphate buffer of pH 6 to 7. An abnormal distribution of areas, similar to that attributed by Krecji, Jennings, and Smith (1941, 1942) and Warner (1944) to α-β interaction, is observed.

The patterns of α-casein (free of κ-casein) over the pH range 3–8 show no marked splitting. Preparations of previous workers showing this heterogeneity probably contain κ-casein. The splitting of the α peak in acid casein is due, at least in part, to the α- and κ-components and their state of aggregation. The patterns of the other fractions examined do not provide further unequivocal evidence of this. The detection of true heterogeneity is further complicated by other interaction effects.

The composition of second-cycle casein—fraction S is also discussed.

I. Introduction

Since Mellander (1939) first observed the resolution of acid casein by moving boundary electrophoresis into the α-, β-, and γ-peaks this method has been used widely to characterize casein preparations. Nevertheless there has been considerable discrepancy between the results of various workers, particularly concerning the behaviour of the α-peak.

Warner (1944) first demonstrated the splitting into two peaks of the ascending α-component of acid casein in veronal buffer at pH 7·8 (*I* 0·1, 0·08M NaCl) when the protein concentration was less than 1·0 per cent. Purified α-casein behaved in a similar manner, and this was confirmed by Cherbuliez and Baudet (1950). Kondo, Yonezawa, and Morita (1950) examined the behaviour of purified α-casein over the pH range 2·5–9·4 (*I* 0·007–0·1) and presented evidence for heterogeneity in both the ascending and descending boundaries under various

* Division of Food Preservation and Transport, C.S.I.R.O., Physico-Chemical Unit, Biochemistry Department, University of Sydney; present address: Department of Physical Biochemistry, Australian National University, Canberra, A.C.T.

† Biochemistry Department, University of Sydney; present address: Department of Biochemistry, Stanford University, California.

conditions. Both Slatter and van Winkle (1952) and Tobias, Whitney, and Tracy (1952) have made an electrophoretic study of diluted dialysed skim milk. The former workers found that α-casein migrated as two distinct peaks, α_1- and α_2-casein, over the pH range 5·4–8·4 (borate, phosphate, and acetate buffers ; I 0·02, 0·1, and 0·05). Tobias, Whitney, and Tracy (1952) noted that in veronal buffer at pH 8·7 (I 0·1) the ascending α component split into three peaks.

Assessment of earlier work on the electrophoresis of casein fractions is difficult owing to the following : (i) unknown variations in the state of purity of the fractions especially with regard to the new component κ-casein ; (ii) the tendency for α-, β-, and κ-casein to form aggregates with themselves and with one another (processes which are highly dependent on pH, ionic strength, and temperature) ; and (iii) the tendency for casein to interact with other proteins and with ions other than the hydrogen ion (this is particularly true in the case of skim milk).

The results of a study of the electrophoretic behaviour of acid casein, various casein fractions, and *pure* α-casein over a limited range of pH, ionic strength and protein concentration are presented here. It will be shown, *inter alia*, that κ-casein is a factor contributing to the heterogeneity commonly described in the " α-peak " of acid casein.

II. Materials and Methods

Buffers : The composition of the buffers used is summarized in Table 1. All chemicals were A.R. grade except the veronal, which was B.P. grade. The pH values given in the electrophoresis results are those of the protein solutions at room temperature and after dialysis.

TABLE 1

COMPOSITION OF BUFFERS

All buffers except phosphate (1) have I 0·02 ; for I 0·1, 16 ml 5M NaCl are added before making up to 1 l.

Buffer	pH	Composition (per litre)
Veronal (1)	9·0	40 ml 0·5M NaV, 5·2 ml 2N HCl
Veronal (2)	8·5	40 ml 0·5M NaV, 2·65 ml 2N HCl
Veronal (3)	8·0	40 ml 0·5M NaV, 1·0 ml 2N HCl
Phosphate (1) (I 0·1)	7·0	1·18 g $NaH_2PO_4.2H_2O$, 2·02 g Na_2HPO_4, 2·92 g NaCl
Phosphate (2) ..	7·0	11·35 ml 0·5M Na_2HPO_4, 0·8 ml 4M NaH_2PO_4
Phosphate (3) ..	6·5	8·3 ml 0·5M Na_2HPO_4, 1·85 ml 4M NaH_2PO_4
Acetate	5·5	10·0 ml 2M NaOAc, 0·6 ml HOAc
Glycine	3·0	15·8 ml M glycine + M NaCl, 2·1 ml 2N HCl

Moving Boundary Electrophoresis : These measurements were made at 0·6 °C in a modified Tiselius cell (Alberty 1949) in a water-bath controlled to within 0·03 °C. After the boundary had been formed, it was brought into view by means of an electrolytic gas compensator, modified from Johnson and Shooter (1949). The optical system was of the Philpot cylindrical lens type. A glass fibre was used instead of the inclined knife-edge. The light-source, horizontal slit, camera lens, and cylindrical lens were of the standard Hilger type. The Hilger camera was modified by removal of the bi-prism in front of the plate holder, and the latter was altered to take a 3¼ by 4¼ in. photographic plate. In earlier work (patterns Nos. 1–8, 10–11) the cylindrical lens was in the normal position. In the later work it was moved to the conjugate position. The low-pressure

mercury arc with a filter isolating the green line was used as light-source. The schlieren lenses were carefully chosen to be free from defects such as spherical aberration, coma, etc. The borosilicate crown glass windows of the water-bath were 2·5 cm thick to minimize distortion due to water pressure and were polished flat to Rayleigh tolerances. The power was supplied by a constant current power supply which could deliver 50 mA at 500 V. The potential drop across a standard 20 Ω resistor was measured with a Leeds and Northrup portable potentiometer at various intervals during each run to obtain an accurate value of the current.

The distance from the starting point to the centroid of each peak was obtained directly from the photographic plate by means of a travelling microscope measuring to within 0·01 mm. Mobilities were calculated from the descending boundaries.

Solutions for electrophoresis (0·5, 0·6, or 1·0% with respect to protein) were prepared by dialysing the protein solution, made up with buffer, against a relatively large volume of the same buffer with stirring for 16 hr at 2 °C. Conductivities of protein solutions were measured at 0 °C in a Shedlovsky type conductivity cell. Area measurements were made from enlarged tracings by means of a planimeter.

All other materials and methods have been described in Part I of this series.*

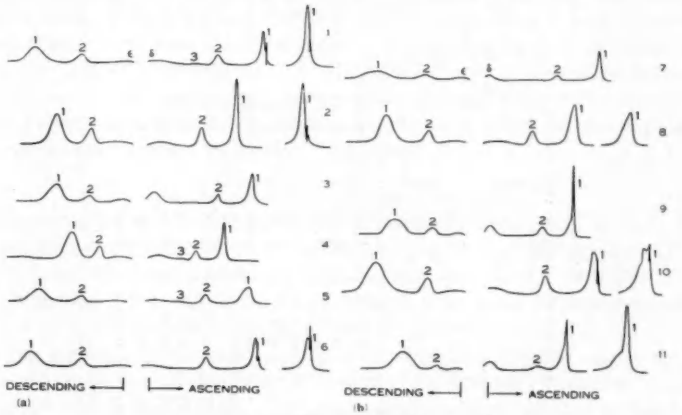


Fig. 1 (*a*) and (*b*).—Electrophoresis of acid casein.

See Table 2 for details. The times after starting the electrophoresis are given below, as well as the angle, θ, made by the inclined glass fibre with the horizontal. The values given in brackets are those for the leading peak at a later time.

1, 251 min, 20° (339 min, 10°); 2, 133 min, 20° (246 min, 15°); 3, 57 min, 40°; 4, 147 min, 30° (asc.), 20° (desc.); 5, 252 min, 20°; 6, 245 min, 20° (300 min, 15°); 7, 72 min, 20°; 8, 209 min, 10°; 9, 66 min, 30°; 10, 242 min, 15° (339 min, 10°); 11, 41 min, 40° (84 min, 20°).

Peaks: 1, "α-" component; 2, β-casein; 3, γ-casein.

III. Results

(*a*) *Acid Casein*

The results of electrophoresis measurements on two different preparations of acid casein (designated I and II) under varying conditions are shown in Table 2 and Figures 1 (*a*) and 1 (*b*). An examination of these patterns indicates some difference in the electrophoretic behaviour of the two preparations.

* See p. 712.

TABLE 2

ELECTROPHORESIS OF ACID CASEIN

Preparation	Pattern No.	Concentration (g/100 ml)	pH and Buffer	I	V/cm	$\mu \times 10^5$ (cm^2 V^{-1} sec^{-1})		Relative Area (%)			
								"α"		β	
						"α"	β	Desc.	Asc.	Desc.	Asc.
I	1	0·6	8·8 Veronal (1)	0·1	3·68	—6·68	—3·40	74	70	26	30
I	2	0·6	8·3 ,, (2)	0·1	3·81	—6·97	—3·40	73	70	27	30
II	3	1·0	8·2 ,, (2)	0·02	7·39	—8·50	—4·46	80	81	20	19
II	4	1·0	8·1 ,, (2)	0·1	3·67	—6·62	—3·16	79	78	21	22
II	5	0·6	7·8 ,, (3)	0·1	3·55	—6·75	—3·30	77	75	23	25
I	6	0·6	7·8 ,, (3)	0·1	3·76	—6·85	—3·20	73	71	27	29
II	7	0·5	7·2 Phosphate (2)	0·02	8·66	—9·92	—4·44	80	79	20	21
II	8	0·5	7·1 ,, (2)	0·1	3·67	—6·84	—2·99	79	75	21	25
I	9	0·6	6·8 ,, (2)	0·02	8·81	—8·71	—3·58	82	71	18	29
I	10	1·0	6·4 ,, (3)	0·1	3·66	—6·39	—2·46	81	70	19	30
II	11	1·0	6·3 ,, (3)	0·02	9·30	—7·50	—2·81	89	82	11	18

In the pH range 6·3–8·8, the ascending α-peak shows signs of splitting only after *prolonged* electrophoresis, both in veronal (pH 7·8–8·8) and phosphate (pH 6·3–7·2) buffers at I 0·02 and 0·1. The " α-peak " is asymmetric with a sharp front, reminiscent of other aggregating systems. In the case of preparation I a sharp spike is also apparent (see Nos. 1, 2, 6), and its position relative to the "α-peak" changes throughout the duration of the experiment.

The proportion of β-casein as determined from the relative areas (per cent.) of the peaks would appear to be slightly higher in preparation I than in preparation II.* For a given preparation and pH the relative area of the β-peak is less in the descending than in the ascending boundary. This is particularly noticeable in the lower pH range (6·3 to 6·8, Nos. 9, 10, 11) where the differences are of the order of 60 per cent. Normally in the absence of interactions it would be expected that the area of the β-peak in the descending limb would be slightly greater (*c.* 5 per cent.) than in the ascending limb. Throughout the pH range studied the relative area of the ascending "α-peak" or of the ascending β-peak is virtually constant. The relative area of the "α-peak" in the descending limb increases while that of the β-peak decreases as the pH falls. There is evidently enhanced interaction as the " α-component " moves down through the medium containing β-casein under the lower pH conditions. Some asymmetry is also noticeable in the descending β-peak, particularly for casein II.

(*b*) *Comparison of Casein Fractions*

The results of an electrophoretic examination of total milk protein, first-cycle casein, second-cycle casein—fractions P and S, and acid casein(III), all prepared from the one pooled raw-milk sample, are shown in Table 3 and Figures 2 (*a*) and 2 (*b*). All these measurements were made in phosphate buffer of pH 7·0 (I 0·02, 0·1) at 1 per cent. protein concentration.

In the patterns of the total milk protein (12,15), first-cycle casein (13,16), and second-cycle casein—fraction P (14,17), the leading ascending peak is asymmetric, with a sharp front. The latter is particularly marked at the lower ionic strength. The descending "α-peak" is asymmetric and shows some tendency to split into two peaks. The total milk protein and first-cycle casein seem to show this effect more than the second-cycle casein—fraction P at I 0·02.

At both ionic strengths the mobility of the leading ("α-κ") peak in first-cycle casein is lower than that of the leading (α) peak in second-cycle casein. This is similar to the result obtained on filter paper electrophoresis, described in Part I of this series. An abnormal distribution of concentrations is again evident. The relative area of the descending β-peak is less than that of the ascending peak. The differences are approximately 40 per cent. in first-cycle casein and 100 per cent. in second-cycle casein—fraction P. These results suggest a greater tendency towards interaction between β- and α-casein than between β-casein and the α-κ complex under these conditions.

Waugh and von Hippel (1956) suggested that the "α-peak" in acid casein represents the two distinct components, α- and κ-casein, involved to a certain

* Although the small γ-casein peak can be detected in some cases, it has been ignored in determining the proportions of the major components.

TABLE 3

ELECTROPHORESIS OF CASEIN FRACTIONS

Protein	Pattern No.	Concentration (g/100 ml)	pH and Buffer	I	V/cm	$\mu \times 10^5$ ($cm^2\ V^{-1}\ sec^{-1}$)		Relative Area (%)			
								α-κ, α, or κ		β	
						α-κ, α, or κ	β	Desc.	Asc.	Desc.	Asc.
Total milk protein ..	12	1·0	7·0 Phosphate (1)	0·1	4·93	—6·24	—2·52	—	—	—	—
1st cycle	13	1·0	7·0 ,, (1)	0·1	5·19	—6·04	—2·43	84	78	16	22
2nd cycle—P ..	14	1·0	7·0 ,, (1)	0·1	4·87	—6·41	—2·57	84	70	16	30
Total milk protein ..	15	1·0	7·0 ,, (2)	0·02	6·85	—8·15	—3·02	—	—	—	—
1st cycle	16	1·0	7·0 ,, (2)	0·02	7·16	—7·62	—2·61	85	78	15	22
2nd cycle—P ..	17	1·0	7·0 ,, (2)	0·02	6·90	—8·05	—2·88	87	72	13	28
Acid casein	18	1·0	7·0 ,, (2)	0·02	7·06	—8·13	—2·86	84	72	16	28
Acid casein*	19	1·0	7·0 ,, (2)	0·02	7·34	—8·08	—3·18	—	—	—	—
2nd cycle—S	20	1·0	7·0 ,, (1)	0·1	4·92	—4·88	—2·46	79	80	21	20
2nd cycle—S	21	1·0	7·0 ,, (2)	0·02	7·20	—	—	—	—	—	—
α-casein	22	0·75	7·0 ,, (2)	0·02	7·24	—8·39	—	—	—	—	—
β-casein	23	0·5	7·0 ,, (2)	0·02	7·14	—	—3·49	—	—	—	—
75% α-casein + 25% β-casein	24	1·0	7·0 ,, (2)	0·02	7·10	—7·62	—2·60	—	—	—	—
1st cycle	25	0·6	7·9 Veronal (3)	0·1	4·08	—6·24	—2·99	—	—	—	—

* Treated at pH 12.

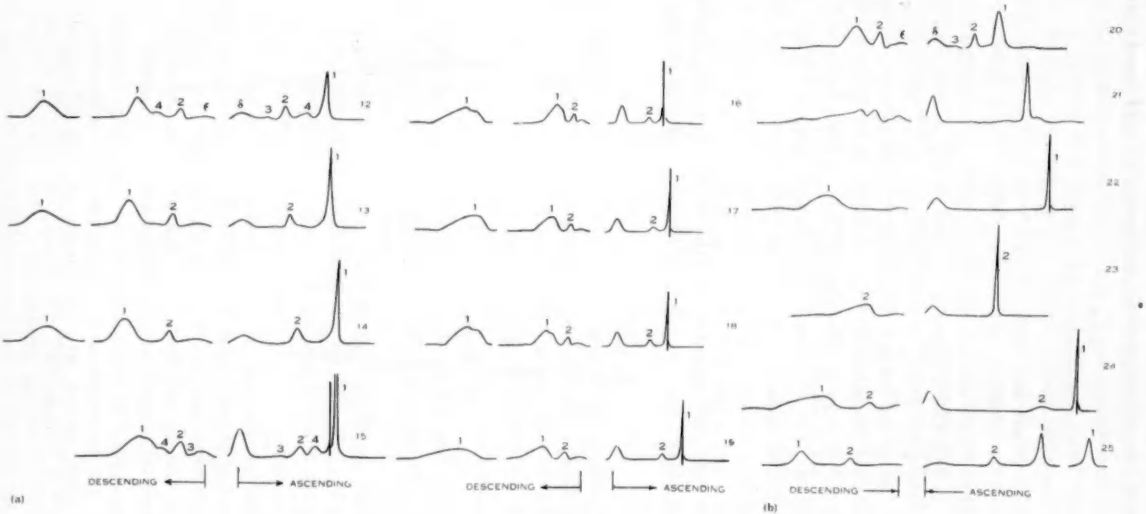


Fig. 2 (*a*) and (*b*).—Electrophoresis of casein fractions. See Table 3 for details.

12, 173 min, 40° (315 min, 30°); 13, 174 min, 40° (340 min, 40°); 14, 176 min, 40° (303 min, 40°); 15, 103 min, 30°; 16, 37 min, 60° (91 min, 40°); 17, 47 min, 60° (88 min, 40°); 18, 42 min, 60° (73 min, 40°); 19, 50 min, 60° (121 min, 40°); 20, 151 min, 40°; 21, 91 min, 40°; 22, 91 min, 40°; 23, 106 min, 40°; 24, 117 min, 40°; 25, 271 min, 40° (331 min, 40°).

Peaks: 1, α-κ-, α-, or κ-casein; 2, β-casein; 3, γ-casein; 4, β-lactoglobulin.

extent in some type of difficultly reversible aggregation. A comparison has therefore been made of the electrophoretic behaviour of acid casein and first-

TABLE 4

ELECTROPHORESIS OF α-CASEIN

Pattern No.	Concentration (g/100 ml)	pH and Buffer	I	V/cm	$\mu \times 10^5$ ($cm^2\ V^{-1}\ sec^{-1}$)
26	0·5	8·2 Veronal (2)	0·1	3·74	—7·17
27	0·6	8·0 ,, (3)	0·1	3·93	—6·95
28	0·6	7·8 ,, (3)	0·1	4·73	—6·60
29	0·6	7·6 ,, (3)	0·1	3·84	—6·60
30	1·0	6·2 Phosphate (3)	0·1	4·81	—6·52
31	1·0	6·2 ,, (3)	0·02	8·68	—8·22
32	1·0	5·6 Acetate	0·02	7·55	—8·56
33	1·0	5·5 ,,	0·1	4·82	—5·99
34	0·5	3·0 Glycine	0·02	6·31	+9·88

cycle casein, from the one milk sample, at pH 7·0 and I 0·02. Under these conditions acid casein (III; No. 18) shows a distinct splitting in the leading

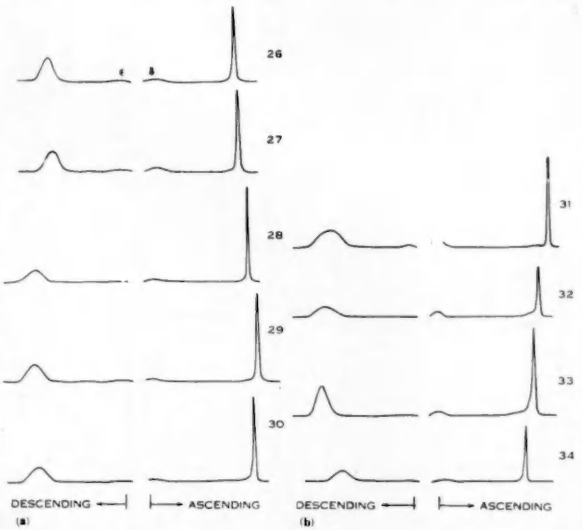


Fig. 3 (*a*) and (*b*).—Electrophoresis of α-casein. See Table 4 for details. 26, 223 min, 12°; 27, 294 min, 40°; 28, 236 min, 40°; 29, 327 min, 30°; 30, 256 min, 50°; 31, 96 min, 40°; 32, 112 min, 60°; 33, 257 min, 40°; 34, 103 min, 40°.

descending peak similar to that obtained with the total milk protein and first-cycle casein. A difference from first-cycle casein can be noted in the higher

mobility of the leading peak, presumably due to a different state of aggregation. It was expected that disaggregation of the complexes involving α- and ϰ-casein by titration of acid casein(III) to pH 12 and subsequent dialysis to pH 7 might result in a pattern still resembling that of first-cycle casein but with similar mobility for the leading peak. The pattern obtained (No. 19), however, resembles more that of second-cycle casein—fraction P, and, in addition, the mobility is still significantly higher than for first-cycle casein.

Electrophoresis of second-cycle casein—fraction S at pH 7·0, I 0·1 (No. 20), shows three peaks. The fastest moving component, with a mobility of $-4{\cdot}88\times10^{-5}$ cm^2 V^{-1} sec^{-1}, accounts for approximately 80 per cent. of the total protein in both the ascending and descending limbs, and represents mainly ϰ-casein. The pattern obtained at I 0·02 (No. 21) is complex.

Patterns for pure α- and β-casein and a mixture of the two are shown in Nos. 22, 23, 24 for comparison purposes. The mixture gives a pattern similar to second-cycle casein—fraction P although the mobilities are somewhat lower.

The result of electrophoresis on first-cycle casein under conditions where the α_1–α_2 split in acid casein has been most commonly described (0·6 per cent. protein, veronal buffer, pH 7·9, I 0·1) is also included (No. 25). There is no evidence of such splitting in this case, even after prolonged electrophoresis.

(*c*) *α-Casein*

The results of an electrophoretic study on α-casein, free from ϰ-casein, are shown in Table 4 and Figures 3 (*a*) and 3 (*b*). Acid casein(II) was used as the source of this α-casein. Over the pH range 3·0–8·2 (I 0·02, 0·1) the pattern shows no marked splitting as is evident in acid casein under similar conditions.

IV. Discussion

Linderstrøm-Lang (1929) and later Sørensen and Sørensen (1939) observed that the composition of acid casein was dependent on the conditions of precipitation—pH, salt concentration, etc. The present work with electrophoretic criteria confirms that there are subtle differences in acid casein preparations. Probably local pH variations during the precipitation and dissolution procedures result in slightly different states of aggregation and proportions of the casein components in the various preparations.

The samples of acid casein used in the present work behave somewhat differently from each other as well as from that examined by Warner (1944). He observed a very distinct splitting of the α-peak after a relatively short time in veronal buffer at pH 7·8 and I 0·1, when the protein concentration was less than 1 per cent. In the present work splitting was observed only after prolonged electrophoresis. However, it occurred even at 1 per cent. concentration and in phosphate buffer of lower pH.

The electrophoretic patterns of the caseins reflect their tendency to aggregation–disaggregation reactions, being similar to those of other associating systems. One such example is β-lactoglobulin studied by Ogston and Tilley (1955), Ogston and Tombs (1957), and McKenzie and Smith (1958). These interactions make detection of true heterogeneity of the casein fractions difficult.

The abnormal distribution of areas, which is a result of "α-"-β-interaction, has also been noticed by Krejci, Jennings, and Smith (1941, 1942) and Warner (1944). The dependence of this interaction on protein concentration is well established (Nitschmann and Zürcher 1950).

The enhanced tendency for the "α-" and β-components of acid casein to interact at the lower pH values, observed here and by other workers (Warner 1944; Slatter and van Winkle 1952), and the more readily distinguishable heterogeneity in the ascending "α-peak" under similar pH conditions suggests a possible connection between the two effects. However, the electrophoretic patterns of pure α-casein (free from κ-casein) obtained in the present work show no marked splitting of the α-peak. On the other hand the results of other workers using α-casein, which almost certainly contained κ-casein, show this splitting. These results suggest that the splitting of the "α-peak" in acid casein is due at least in part to the α- and κ-components. Unfortunately the differences in patterns of first-cycle casein and second-cycle casein—fraction P are not sufficiently great to provide further unequivocal evidence of this. Because no distinct splitting can be observed in the leading ascending peaks of the total milk protein and first-cycle casein, even under conditions where it has been most commonly described for acid casein, it is possible that such splitting is due not directly to κ-casein but to its incorporation into various "difficultly reversible aggregates" with α-casein on acid precipitation.

The asymmetry in the peaks and the tailing in the patterns of pure α-casein (more noticeable at the lower pH values above the isoelectric point) is probably due to aggregation–disaggregation of α- and the presence of relatively large aggregates which form as the pH is lowered. A preparation of α-casein in glycine–HCl buffer at pH 3·0, I 0·1, showed extensive aggregation even at 0·5 per cent. protein. The solution was quite milky and it is possible that the heterogeneity described by Warner (1944) for α-casein in lactate buffer under similar conditions was due to such aggregation.

Waugh and von Hippel (1956) suggested that second-cycle casein—fraction S contains 70 per cent. κ-casein and 30 per cent. β-casein. In the present work moving boundary electrophoresis of second-cycle casein—fraction S at pH 7·0, I 0·1, has indicated the presence of approximately 80 per cent. of a major component. Paper electrophoresis and sedimentation of fraction S, as well as results on the action of rennin on this material, indicate a much lower proportion of κ-casein, nearer 50 per cent. (see Parts I and V of this series). The complexity of the pattern obtained at pH 7·0, I 0·02, is probably a result of various types of interactions involving κ-casein and possibly other components. The minor components in this fraction have been discussed in Part I.

Mention should be made here of the recent work of Payens (1958). He has reported briefly the results of an electrophoretic study of the action of commercial rennet on first-cycle casein and second-cycle casein—fraction P. He draws the conclusion that the commonly described "α-casein" of the α_1-α_2 split is closely related to κ-casein and is possibly identical with it. In veronal buffer at pH 7·3, I 0·1, 1·7 per cent. first-cycle casein occasionally exhibited the α_1-α_2 split in the ascending limb. Payens' (1958) results, showing the

presence of 27 per cent. α_2-casein in first-cycle casein, indicates that α_2-casein is not identical with $\varkappa$-casein. Results presented in Part V of the present series support Waugh and von Hippel's estimation of only 15 per cent. $\varkappa$-casein in first-cycle casein. Also, it will be apparent that second-cycle casein—fraction P contains practically no $\varkappa$-casein. The appearance of 13 per cent. α_1-casein (unresolved) in the ascending limb during electrophoresis of Payens' second-cycle casein—fraction P, therefore, cannot be explained by the presence of $\varkappa$-casein.

It is obvious that work over a wide range of pH, temperature, ionic strength, and protein concentration is necessary to throw further light on the electrophoretic heterogeneity of casein.

V. Acknowledgments

In addition to those acknowledged in Part I, grateful acknowledgment is due to Dr. R. G. Giovanelli and the National Standards Laboratory, Division of Physics, C.S.I.R.O., for making available and testing the schlieren lenses.

VI. References

Alberty, R. A. (1949).—*J. Phys. Chem.* **53**: 114.
Cherbuliez, E., and Baudet, P. (1950).—*Helv. Chim. Acta* **33**: 398.
Johnson, P., and Shooter, E. M. (1949).—*Science* **109**: 39.
Kondo, K., Yonezawa, T., and Morita, Y. (1950).—*Bull. Res. Inst. Food Sci. Kyoto Univ.* **31**: 40.
Krecji, L. E., Jennings, R. K., and Smith, L. P. (1941).—*J. Franklin Inst.* **232**: 592.
Krecji, L. E., Jennings, R. K., and Smith, L. P. (1942).—*J. Franklin Inst.* **234**: 197.
Linderstrøm-Lang, K. (1929).—*C.R. Lab. Carlsberg* (ser. chim.) **17** (9): 1.
McKenzie, H. A., and Smith, M. B. (1958).—Paper read to Adelaide Meeting of A.N.Z.A.A.S. August 1958.
Mellander, O. (1939).—*Biochem. Z.* **300**: 240.
Nitschmann, H., and Zürcher, H. (1950).—*Helv. Chim. Acta* **23**: 1648.
Ogston, A. G., and Tilley, J. M. A. (1955).—*Biochem. J.* **59**: 644.
Ogston, A. G., and Tombs, M. (1957).—*Biochem. J.* **66**: 399.
Payens, T. A. J. (1958).—*Nature* **181**: 114.
Slatter, W., and van Winkle, Q. (1952).—*J. Dairy Sci.* **35**: 1083.
Sørenson, M., and Sørenson, S. P. L. (1939).—*C.R. Lab. Carlsberg* (ser. chim.) **23** (7): 1.
Tobias, J., Whitney, R. M., and Tracy, P. H. (1952).—*J. Dairy Sci.* **35**: 1036.
Warner, R. C. (1944).—*J. Amer. Chem. Soc.* **66**: 1725.
Waugh, D. F., and von Hippel, P. H. (1956).—*J. Amer. Chem. Soc.* **78**: 4576.

STUDIES OF CASEIN

III. THE MOLECULAR SIZE OF α-, β-, AND κ-CASEIN

By H. A. McKenzie* and R. G. Wake†

[*Manuscript received January* 30, 1959]

Summary

α-, β-, and κ-casein are aggregated at neutral pH and room temperature in salt solution. They may be disaggregated at high pH or at neutral pH in concentrated urea solution.

At neutral pH in salt solution, α-casein forms aggregates centring around a preferred size. As the pH is increased the size decreases until at pH 11 (I 0·20) it is disaggregated completely. Measurements of the molecular weight by sedimentation and diffusion at pH 11 give a value of 24,800±1000. Approach to sedimentation-equilibrium measurements at pH 12, using the Archibald method, gives a value of 25,500±1000. In 6M urea solution at pH 7·3 sedimentation and diffusion give a value of 27,600±1000.

At room temperature and neutral pH, β-casein is present as single molecules in equilibrium with an aggregate of very high molecular weight. At pH 11, it is disaggregated with a molecular weight of 17,300±800 (by sedimentation-diffusion). At pH 7 in 6M urea a value of 19,800±1000 is obtained for the molecular weight.

Preliminary measurements by the Archibald method at pH 12 give a value of 26,000±3000 for the molecular weight of κ-casein.

These results are discussed in relation to those of other workers.

I. Introduction

At the present time there is considerable disagreement between the results of various workers concerning the molecular size of α- and β-casein. Svedberg, Carpenter, and Carpenter (1930) first estimated the molecular weight of the major sedimenting component in acid casein to be 75,000–100,000. Burk and Greenberg (1930) found from osmotic pressure measurements that the molecular weight of whole casein in 6·66M urea was 33,600. Hipp *et al.* (1952) showed that urea brings about a dispersion of casein aggregates. D'yachenko and Vlodavets (1952) showed that disaggregation could also be effected by extremes of pH.

The molecular weights of importance are those determined for the individual components of casein. Here again the results obtained by various workers using "pure" fractions differ. This can be partly explained by the ease with which α- and β-casein aggregate. In addition, the α-casein used by workers before 1956 could have been contaminated with the recently discovered κ-casein.

* Division of Food Preservation and Transport, C.S.I.R.O., Physico-Chemical Unit, Biochemistry Department, University of Sydney; present address: Department of Physical Biochemistry, Australian National University, Canberra, A.C.T.

† Biochemistry Department, University of Sydney; present address: Department of Biochemistry, Stanford University, California.

Cherbuliez and Baudet (1950) have estimated the minimum molecular weight of their α- and β-casein, on the basis of tyrosine, tryptophan, and phosphorus content, to be 130,000 and 48,000 respectively. From the phosphorus content of α-casein, Perlmann (1954) suggested a minimum molecular weight of 31,000. A study of the influence of temperature on the apparent size of α- and β-casein has been made recently by Sullivan *et al.* (1955). Sedimentation and diffusion experiments at room temperature and at 8 °C revealed essentially one component in α-casein at pH 7·8 with a molecular weight of 121,800. At room temperature and neutral pH β-casein showed two distinct sedimenting peaks. Lowering the temperature produced a shift in the relative amounts of the two components, and at 15 °C the faster-moving one was completely absent. The molecular weight at low temperature was calculated to be 24,100. Halwer (1954) found the aggregation of α- and β-casein was electrolyte concentration dependent at neutral pH and that they resembled the denatured forms of globular proteins. He was unable to reach any conclusion about their molecular size.

An examination of the sedimentation behaviour of second-cycle casein—fraction P which is essentially a mixture of α- and β-casein, has been made by von Hippel and Waugh (1955). On the basis of this study it was suggested that the molecular weight of α-casein lies in the range of 13,000–15,000, and that of β-casein in the range 15,000–25,000.

In the present paper a study is made of the behaviour of pure α- and β-casein during sedimentation under various conditions. α-Casein, free from κ-casein, has been used throughout. The molecular weights have been calculated under conditions favouring disaggregation of these two proteins in an attempt to determine their molecular weights. Some preliminary measurements of the molecular size of κ-casein are also reported.

II. Materials and Methods

Protein Preparations : α-Casein free of κ-casein was prepared by alcohol fractionation (see Parts I and II of this series). β-Casein was prepared by the alcohol method of Hipp *et al.* (1952). κ-Casein was prepared as described in Part IV of this series.

Protein Concentrations : These were obtained from Kjeldahl nitrogen determinations carried out according to the procedure of McKenzie and Wallace (1954) or from dry weight estimations. The percentages of nitrogen in α- and β-casein were taken as 15·6 and 15·3 respectively.

Reagents and Buffers : The urea (C.P.) was recrystallized once from aqueous alcohol. The stock phosphate buffer (0·5M), used with the 6M urea solutions, was prepared by mixing 0·5M Na_2HPO_4 and 0·5M KH_2PO_4 in the required proportion, and the borate buffer (0·5M) from 0·5M H_3BO_3 and 0·5N NaOH. The pH 12·0 phosphate buffer (I 0·19) was made by dissolving 7·1 g Na_2HPO_4 and 1·33 g NaOH in 1 l. water. The pH 11·0 glycine buffer (I 0·20) was prepared by diluting the following to 1 l. with water : 10·2 ml N NaOH, 9·8 ml 1·0M glycine–1·0M NaCl, and 36 ml 5·0M NaCl.

Sedimentation Velocity : Measurements of sedimentation rates were carried out at room temperature (19–24 °C) in a Spinco Model E analytical ultracentrifuge at a nominal speed of 59,780 r.p.m. An analytical cell with a 12 mm thick centrepiece (Kel-F for pH's >10) was used. An inclined wire replaced the bar in the Philpot cylindrical lens optical system. Rotor temperature was measured at the beginning and end of a run. After application of the Waugh and Yphantis (1952) correction, the temperature at any time was found by interpolation.

A travelling microscope measuring to within 0·01 mm was used to determine distances. The distance from the axis of rotation to the inner reference line was taken as 5·725 cm at

59,780 r.p.m. (Taylor 1952). Sedimentation coefficients (s) were calculated by the method of Cecil and Ogston (1948). All values of s are given in Svedbergs and refer to the solvent in which the determinations were made, unless otherwise stated.

Approach to Sedimentation Equilibrium : The Archibald ultracentrifugal procedure was applied to α-casein at pH 12 (Archibald 1947). The ultracentrifuge was run at room temperature and a nominal speed of 12,590 r.p.m. over a period of approx. 800 min. The initial and final temperatures of the rotor differed by about 1·5 °C and the mean value was used in subsequent calculations. For three consecutive runs the average temperature varied between 19·3 and 19·6 °C. The concentration of protein at the meniscus of the cell, at a distance r_m from the axis of rotation, was calculated using the simplified procedure of Charlwood (1957). The concentration at the bottom of the cell, C_b, was not determined since the use of an artificial cell base of an immiscible liquid, such as a silicone, was considered undesirable in view of the general properties of casein. The concentration differences were obtained from area measurements on patterns enlarged approximately seven times. The area corresponding to a known concentration difference, with the inclined wire at different angles, was determined on a 0·82% α-casein solution at neutral pH using the synthetic boundary cell.

Molecular weights have been calculated from δm, using the relation,

$$\delta m = \frac{1}{r_m C_m} \cdot \left(\frac{dC}{dr}\right)_{r_m} = \frac{M(1-\bar{v}\rho)\omega^2}{RT},$$

where M is the molecular weight,
ω is the angular velocity,
$\bar{v}$ is the partial specific volume,
ρ is the density of solvent,
R is the gas constant,
T is the absolute temperature.

Preliminary measurements were also made by the Archibald procedure for κ-casein at pH 12. The ultracentrifuge was run at a nominal speed of 15,220 r.p.m. The average temperatures for centrifugations at initial protein concentrations of 0·27, 0·54, and 0·80% (determined by ultraviolet absorption) varied between 21·1 and 21·3 °C. A run in the synthetic boundary cell at pH 9 (0·02M $Na_2B_4O_7$, 0·15M NaCl) was carried out in order to relate concentration differences with areas under the curve at various angles of the inclined wire in the schlieren optical system. Measurements were also made at 0·48% protein (by Kjeldahl) using a Spinco phase plate.

Diffusion : Diffusion measurements were made at 25 °C in a stainless steel Claesson-type diffusion cell in a water-bath controlled to within 0·03 °C. The optical system was of the Philpot cylindrical lens type and has been described in Part II of this series.

The solutions for analysis were prepared by dialysing a small amount of protein-buffer solution against a relatively large volume of the same buffer with stirring for 24 hr at 25 °C. Precautions were taken while filling the cell to prevent evaporation from the solutions ; this was particularly important where concentrated urea solutions were used. The filled cell was allowed to equilibrate for at least 1 hr before forming the boundary. Exposures were then taken at suitable intervals over a period of 3–4 days.

For calculations the diffusion curves were enlarged approximately 15 times. Weight average coefficients, D, were determined by the method of moments. Values of D are given in c.g.s. units $\times 10^7$.

Molecular weights using D values were calculated from s and D extrapolated to zero concentration. The Svedberg relation, $M = RTs/D(1-\bar{v}\rho)$, was used.

Partial Specific Volumes ($\bar{v}$) *:* McMeekin, Groves, and Hipp (1949) found no change in the partial specific volume of unfractionated casein in concentrated urea solution. McKenzie, Smith, and Wake (1955) and Charlwood (1957) found only small changes in $\bar{v}$ for other proteins in urea solution. Considering the overall accuracy of the present methods the values of $\bar{v}$ for the native proteins were used. These were 0·728 and 0·741 at 25 °C for α- and β-casein respectively.

The density of the aqueous urea solutions was obtained from the data of Gucker, Gage, and Moser (1938).

III. Results

(*a*) *α-Casein*

Two preparations of α-casein (designated I and II) were examined in the ultracentrifuge. Both preparations were free of β-casein by the electrophoretic criterion. At pH 6·8 in 0·1M NaCl and at room temperature the sedimentation

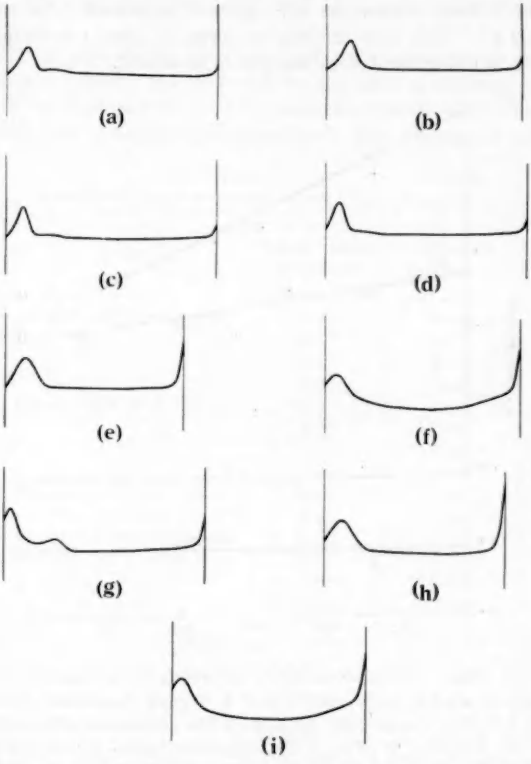


Fig. 1.—Sedimentation of α- and β-casein under various conditions. All runs were carried out with 0·5 per cent. protein at approximately 20 °C. The times and inclined-wire angles are indicated below. Sedimentation is from left to right.

(*a*) α-casein-I, pH 6·8, 18 min, 75°; (*b*) α-casein-II, pH 6·8, 17 min, 70°; (*c*) α-casein-I, pH 7·7, 17 min, 75°; (*d*) α-casein-I, pH 9·7, 19 min, 75°; (*e*) α-casein-I, pH 11·0, 80 min, 75°; (*f*) α-casein-I in 6M-urea, pH 7·3, 82 min, 75°; (*g*) β-casein, pH 6·9, 18 min, 60°; (*h*) β-casein, pH 11·1, 86 min, 70°; (*i*) β-casein in 6M-urea, pH 7·2, 90 min, 70° (see text for other details).

pattern of α-casein(I) exhibited a main peak with s_{20} 4·4. There was a small amount of fast moving material with s_{20} 9, as shown in Figure 1 (*a*). The second preparation, α-casein(II), did not show the fast moving peak (Fig. 1 (*b*)). The

value of s_{20} 4·4 is in close agreement with the value obtained by von Hippel and Waugh (1955) for the α-component of second-cycle casein—fraction P.

As the pH was increased the s_{20} value for α-casein decreased. At pH 7·7 (0·1M NaCl), 9·7 (0·05M NaCl, 0·05M borate), and 11 (glycine buffer, I 0·2) the corresponding s_{20} values, at 0·5 per cent. protein concentration, were 3·8, 2·7, and 1·3 respectively. Decreasing amounts of the faster moving material in preparation I were present as the pH was increased. No trace of it was present at pH 11. This fast moving material is almost certainly aggregated α-casein which is disaggregated as the pH is increased.

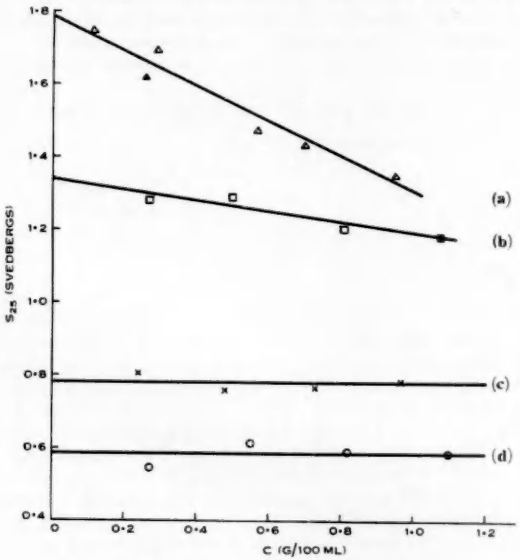


Fig. 2.—Dependence of s_{25} on protein concentration.
(*a*) α-casein-I, glycine buffer, pH 11·0, I 0·20; (*b*) β-casein-I, glycine buffer, pH 11·1, I 0·20; (*c*) α-casein-I, 6M urea, 0·05M phosphate buffer, 0·05M NaCl, pH 7·3; (*d*) β-casein, 6M urea, 0·05M phosphate buffer, 0·05M NaCl, pH 7·2. The lines shown for (*a*) and (*b*) are the relevant regression lines of s on C. Those for (*c*) and (*d*) are mean values of s.

In view of the general aggregation properties of proteins, and casein in particular, observed in this laboratory over a number of years, it was considered that α-casein is probably completely disaggregated at pH 11. Therefore sedimentation and diffusion measurements were carried out at different protein concentrations in glycine buffer at pH 11·0 (I 0·2) in an attempt to determine the molecular weight. The sedimentation results are shown in Figure 2 and and those for diffusion in Table 1.

It will be noted that in accordance with general expectation there is little, if any, dependence of diffusion coefficient on protein concentration. Accordingly,

in calculating the molecular weight the mean value of D has been taken as the D^0 value (i.e. D at zero-protein concentration). On the other hand there is an appreciable dependence of s, for α-casein, on protein concentration. The calculated regression line ($s=s_0-bc$) gives a value of s^0_{25} of $1\cdot78_4$ ($\pm0\cdot03_2$) ($b=0\cdot47_8\pm0\cdot05_7$). Using the latter value, with a D^0_{25} of $6\cdot65\pm0\cdot08$, a molecular weight of 24,800 is obtained. (The accuracy of M values in this paper is probably ±4 per cent.) The corresponding $s_{20,w}$ and $D^0_{20,w}$ values are 1·61 and 5·91 respectively.

Casein may be effectively disaggregated by concentrated urea solution. (The urea does not "split" the molecule as has been sometimes mentioned in the literature (cf. McMeekin 1954).) Urea solution, therefore, provides a suitable medium for molecular weight determination. The results of sedimentation

TABLE 1

DIFFUSION COEFFICIENT VALUES FOR α- AND β-CASEIN

Protein	Solvent	Concentration (g/100 ml)	$D_{25}\times10^7$ (cm² sec⁻¹)	Mean
α-Casein ..	Glycine, pH 11·0, I 0·20	0·29	6·70	
		0·57	6·75	
		0·67	6·75	
		0·95	6·40	6·65
β-Casein ..	Glycine, pH 11·1, I 0·20	0·25	7·65	
		0·50	7·30	
		0·75	7·40	
		1·00	7·70	7·51
α-Casein ..	6M urea, 0·05M phosphate,	0·48	3·45	
	0·05M NaCl, pH 7·3	0·97	3·40	3·43
β-Casein ..	6M urea, 0·05M phosphate,	0·55	3·85	
	0·05M NaCl, pH 7·2	0·55	3·75	
		0·82	4·10	
		0·82	3·80	3·88

and diffusion measurements for α-casein(I) in 6M urea at pH 7·3 (0·05M phosphate, 0·05M NaCl) are shown in Figure 2 and Table 1. A typical sedimentation pattern is shown in Figure 1 (f). There is negligible dependence of s on C within the precision of the measurements and the mean s value of $0\cdot77_9\pm0\cdot01_0$ has been taken as s^0_{25}. D is also independent of C and D^0_{25} is taken as $3\cdot43\pm0\cdot10$. These values give an M of 27,600. The $s_{20,w}$ and $D^0_{20,w}$ values are 1·33 and 4·33 respectively.

The molecular weight obtained above is approximately double that suggested by von Hippel and Waugh (1955) from studies of second-cycle casein—fraction P at pH 12·0. It was also noted in the present work that s_{25} for α-casein (1·0 per cent.) decreases to 1·1 if the pH is raised to 12·0 (phosphate I 0·19). Accordingly, an attempt was made to estimate the molecular weight under these conditions. Use was made of the Archibald procedure for this determination. Values for δm and C_m obtained for α-casein(II) are shown in Table 2. It will be noted that there is no appreciable dependence of δm on time, but that δm is concentration

dependent. Accordingly δm was extrapolated to zero concentration by obtaining the appropriate regression line. This gave a $(\delta m)_{c=0}$ value of $0{\cdot}48_9 \pm 0{\cdot}01_2$ from which a value of M of 25,500 was obtained. This is in satisfactory agreement with the values obtained from sedimentation-diffusion measurements.

TABLE 2

ARCHIBALD ULTRACENTRIFUGAL PROCEDURE APPLIED TO α-CASEIN AT pH 12·0 (I 0·19)—VALUES OF δm AND C_m

Time (min)	Initial Protein Concentration (g/100 ml)					
	0·39		0·68		1·05	
	δm	C_m	δm	C_m	δm	C_m
312	0·39	0·303	0·33	0·551	0·25	0·889
513	0·41	0·284	0·32	0·526	0·24	0·857
560	0·42	0·272	0·30	0·522	0·24	0·850
596	0·43	0·271	0·30	0·519	0·25	0·835
660	0·42	0·268				
716	0·40	0·267				

(*b*) β-*Casein*

The sedimentation pattern for β-casein at room temperature and pH 6·9 (0·5 per cent. protein, 0·1M NaCl) is shown in Figure 1 (*g*). A considerable amount of fast moving material with $s_{20}=11{\cdot}5$ is present. The major slow moving component has $s_{20}=1{\cdot}5$. On increasing the pH to 11·0 (glycine, I 0·20) only one peak of $s_{20}=1{\cdot}5$ separates (see Fig. 1 (*h*)).

The results of sedimentation and diffusion studies in glycine buffer at pH 11·0 (I 0·20) are given in Figure 2 and Table 1. It will be noted that the concentration dependence of s for β-casein is less than for α-casein ($b=0{\cdot}146 \pm 0{\cdot}04_3$). The s^0_{25} value is $1{\cdot}33_8 \pm 0{\cdot}03_2$ ($s^0_{20,w}=1{\cdot}22$). The D^0_{25} value is $7{\cdot}51 \pm 0{\cdot}10$ ($D^0_{20,w}=6{\cdot}67$), from which a molecular weight of 17,300 is computed.

The sedimentation pattern of β-casein in 6M urea at pH 7·2 (0·05 phosphate, 0·05M NaCl) shows only a single slow moving peak (Fig. 1 (*i*)). Sedimentation and diffusion measurements under these conditions give $s^0_{25}=0{\cdot}58_6 \pm 0{\cdot}01_4$ ($s^0_{20,w}=1{\cdot}02$) and $D^0_{25}=3{\cdot}88 \pm 0{\cdot}08$ ($D^0_{20,w}=4{\cdot}89$). The molecular weight is computed as 19,800.

(*c*) κ-*Casein*

The marked aggregation of κ-casein at neutral pH and its disaggregation at high pH are discussed in Part IV of this series. Preliminary measurements by the Archibald method were made at pH 12 in an attempt to determine the molecular weight. The values of δm obtained over the period 64–320 min showed no apparent gradation during the course of a run and thus there was no indication of polydispersity under these conditions. As with α-casein, δm was concentration dependent. Assuming the partial specific volume of κ-casein is 0·73, a preliminary value of the molecular weight of 26,000 ±3000 was obtained.

IV. Discussion

In recent years a number of investigators have studied factors affecting the association and aggregation of α- and β-casein. It is generally agreed that at room temperature and neutral pH, in the presence of salt, both α- and β-casein are aggregated. Sullivan *et al.* (1955) and McMeekin and Peterson (1955) have shown that β-casein is disaggregated as the temperature is lowered, whereas α-casein is unaffected. McMeekin and Peterson (1955) and von Hippel and Waugh (1955) have shown that the aggregates of these two proteins dissociate in alkaline solution. Peterson (1955) and Wake (1955) have reported the presence of disaggregated casein components in concentrated urea solution. The effect of salt concentration on extent of aggregation has been noted by Halwer (1954) and McMeekin and Peterson (loc. cit.). This previous work is in general qualitative agreement with the observations of the present authors.

The fact that mainly single, well-defined peaks with decreasing s values are observed in α-casein as the pH is increased indicates that at each pH there are aggregates centring around a preferred size, which decreases with increasing pH.

The values of 24,800 obtained for the molecular weight of α-casein from sedimentation and diffusion measurements at pH 11·0 and that of 25,500 by the Archibald method at pH 12·0, are in good agreement. The lowering of the sedimentation rate as the pH is increased from 11 to 12 is evidently not due to a change in molecular size but must result from alteration in the shape characteristics of the molecule. The molecular weight of 27,600 at pH 7 in 6M urea is slightly higher and this may be due to method of calculation, differences in partial specific volume, etc. (*vide infra*). Both values are considerably higher than the value of 13,000–15,000 suggested by von Hippel and Waugh (1955). It is understood that recent measurements by Gillespie and Waugh (unpublished data, but quoted by Waugh at the meeting of the Faraday Society, April, 1958) give a value of 23,000. The value of 121,800 reported by Sullivan *et al.* (1955) at pH 7·8 is undoubtedly due to a polymer containing four or five α-casein molecules.

The behaviour of β-casein on sedimentation at pH 6·9 and room temperature, showing the presence of very large polymer, is in accord with the observations made by Sullivan *et al.* (1955) and McMeekin and Peterson (1955). The s_{20} value of 1·5 obtained by us for the slow moving material is essentially the same as that obtained for single molecules of β-casein at low temperature by Sullivan *et al.* The value of 24,100 obtained by the latter (s, D at low temperature) for the molecular weight of β-casein is somewhat higher than the two values 17,300 and 19,800 obtained in the present work. Both results are in the range of 15,000–25,000 suggested by von Hippel and Waugh (1955).

The value of 26,000 obtained for κ-casein in the present work is considerably higher than that of 16,000 obtained by Gillespie and Waugh (unpublished data, but quoted by Waugh at the meeting of the Faraday Society, April 1958). The reason for this discrepancy is not at present obvious.

The molecular weight values obtained in the present work are considered to be reliable. Nevertheless, work is at present in progress to obtain more precise values, particularly for κ-casein. At the same time precise measurements of partial specific volume in a variety of solvents are being made.

V. REFERENCES

ARCHIBALD, W. J. (1947).—*J. Phys. Chem.* **51**: 1204.

BURK, N. F., and GREENBERG, D. M. (1930).—*J. Biol. Chem.* **87**: 197.

CECIL, R., and OGSTON, A. G. (1948).—*Biochem. J.* **43**: 592.

CHARLWOOD, P. A. (1957).—*Trans. Faraday Soc.* **53**: 871.

CHERBULIEZ, E., and BAUDET, P. (1950).—*Helv. Chim. Acta* **33**: 398.

D'YACHENKO, P. F., and VLODAVETS, I. N. (1952).—*Kolloid. Zh.* **14**: 338.

GUCKER, F. T., GAGE, F. W., and MOSER, C. E. (1938).—*J. Amer. Chem. Soc.* **60**: 2583.

HALWER, M. (1954).—*Arch. Biochem. Biophys.* **51**: 79.

HIPP, N. J., GROVES, M. L., CUSTER, J. H., and MCMEEKIN, T. L. (1952).—*J. Dairy Sci.* **35**: 272.

VON HIPPEL, P. H., and WAUGH, D. F. (1955).—*J. Amer. Chem. Soc.* **77**: 4311.

MCKENZIE, H. A., SMITH, M. B., and WAKE, R. G. (1955).—*Nature* **176**: 738.

MCKENZIE, H. A., and WALLACE, H. S. (1954).—*Aust. J. Chem.* **7**: 55.

MCMEEKIN, T. L. (1954).—"The Proteins." Vol. 2, Part A. (Eds. H. Neurath and K. Bailey.) (Academic Press: New York.)

MCMEEKIN, T. L., GROVES, M. L., and HIPP, N. J. (1949).—*J. Amer. Chem. Soc.* **71**: 3298.

MCMEEKIN, T. L., and PETERSON, R. F. (1955).—Abstr. 128th Meeting American Chemical Society, Minneapolis. p. 2A.

PERLMANN, G. E. (1954).—*Biochim. Biophys. Acta* **13**: 452.

PETERSON, R. F. (1955).—Abstr. 128th Meeting American Chemical Society, Minneapolis. p. 21C.

SULLIVAN, R., FITZPATRICK, M., STANTON, E., ANNINO, R., KISSEL, G., and PALERMITI, F. (1955).—*Arch. Biochem. Biophys.* **55**: 455.

SVEDBERG, T., CARPENTER, L. M., and CARPENTER, D. C. (1930).—*J. Amer. Chem. Soc.* **52**: 241.

TAYLOR, T. (1952).—*Arch. Biochem. Biophys.* **36**: 411.

WAKE, R. G. (1955).—Thesis, University of Sydney.

WAUGH, D. F., and YPHANTIS, D. A. (1952).—*Rev. Sci. Instrum.* **23**: 609.

SHORT COMMUNICATIONS

THE NEAR INFRA-RED ABSORPTION OF NORMAL ALCOHOLS AND THEIR BROMIDES*

By R. J. W. Le Fèvre,† R. Roper,† and A. J. Williams†

Specimens of *cyclo*pentanol, *cyclo*hexanol, 10 *n*-alcohols, and the corresponding bromides have recently been purified for examination of various physical properties, among which the near infra-red spectra have been included. Observations (recorded on a Perkin-Elmer spectracord, model 4000) are given in Tables 1 and 2.

TABLE 1

NEAR INFRA-RED SPECTRA OF *n*-ALCOHOLS

Showing $\lambda_{max.}$ and, in parentheses below $\lambda_{max.}$, transmittance

Methanol	Ethanol	*n*-Propanol	*n*-Butanol	*n*-Amyl	*n*-Hexanol
0·901	0·894	0·900	0·910	0·910	0·910
(0·830)	(0·830)	(0·830)	(0·810)	(0·820)	(0·775)
1·005	1·005	1·005	1·010	1·015	1·015
(0·760)	(0·790)	(0·810)	(0·810)	(0·830)	(0·810)
—	—	—	—	—	1·170 sh
					(*c.* 0·20)
1·189	1·182	1·182	1·190	1·195	1·187
(0·280)	(0·240)	(0·230)	(0·200)	(0·200)	(0·160)
1·240	1·200 sh	—	—	—	—
(0·580)	(0·36)				
—	—	—	—	—	1·360
					(0·220)
—	—	—	1·400	1·400	1·385
			(0·120)	(0·140)	(0·120)
→					←
1·580	1·570	1·580	1·580	1·580	1·570
(0·360)	(0·520)	(0·575)	(0·622)	(0·670)	(0·687)
1·690	1·685	—	—	—	—
(0·375)	(0·410)				
1·710 sh	1·720	—	1·710	1·710	1·700
(*c.* 0·41)	(0·435)		(0·360)	(0·360)	(0·320)
—	1·745 sh	1·730 sh	1·74 sh	1·740	1·740
	(0·55)	(0·46)	(0·462)	(0·465)	(0·430)
—	1·80 br.sh	1·80 sh	1·80 sh	1·80 br.sh	1·80 br.sh
	(0·67)	(0·65)	(0·65)	(0·64)	(0·63)
1·940	1·938	*c.* 1·93 sh	1·94 sh	1·94	—
(0·440)	(0·670)	(0·71)	(0·68)	(0·73)	
2·070	2·080	2·082	2·076	2·085	2·070
(0·165)	(0·234)	(0·290)	(0·340)	(0·400)	(0·420)
2·270	2·265	—	—	—	—
(0·010)	(0·020)				

→ All observations below this rule taken in 1 mm cells, those above, in 1 cm cells.

* Manuscript received May 29, 1959.

† School of Chemistry, University of Sydney.

TABLE 1 (*Continued*)

Methanol	Ethanol	*n*-Propanol	*n*-Butanol	*n*-Amyl	*n*-Hexanol
—	2·300	2·295	2·296	2·295	2·290
	(0·010)	(0·010)	(0·020)	(0·020)	(0·010)
—	2·345	2·340	—	2·330	2·330
	(0·056)	(0·050)		(0·035)	(0·020)
—	—	2·385	2·390	—	—
		(0·060)	(0·055)		
—	2·47 br	2·47 br	2·450	2·450	2·450
	(0·035)	(0·040)	(0·040)	(0·042)	(0·036)
—	—	—	2·50	2·500	2·495
			(0·070)	(0·092)	(0·076)
—	2·590	2·60	2·60	2·575	2·560
	(0·230)	(0·195)	(0·178)	(0·180)	(0·170)

n-Octanol	*n*-Nonanol	*n*-Decanol	*n*-Dodecanol	*cyclo*Pentanol	*cyclo*Hexanol
0·918	0·915	0·920	0·915	0·900	0·910
(0·770)	(0·750)	(0·816)	(0·780)	(0·810)	(0·810)
1·020	1·020	1·030	1·020	1·010	1·020
(0·815)	(0·800)	(0·850)	(0·820)	(0·825)	(0·850)
—	—	—	—	1·170	—
				(0·150)	
1·190	1·190	1·200	1·190	—	1·190
(0·156)	(0·145)	(0·150)	(0·132)		(0·145)
—	—	—	—	—	—
1·370	1·370	—	1·38 sh	1·360	1·370
(0·210)	(0·222)		(0·235)	(0·320)	(0·310)
1·390	1·390	1·400	1·390	1·400	1·400
(0·120)	(0·136)	(0·165)	(0·165)	(0·150)	(0·170)
→					←
1·570	1·580	1·580	1·580	1·580	1·580
(0·742)	(0·73)	(0·770)	(0·805)	(0·650)	(0·700)
—	—	—	—	—	—
1·710	1·710	1·720	1·715	1·690	1·712
(0·320)	(0·300)	(0·325)	(0·313)	(0·315)	(0·290)
1·745	1·740	1·750	1·748	1·730	1·740
(0·430)	(0·410)	(0·440)	(0·435)	(0·330)	(0·335)
1·78	1·78	1·79 sh	1·78 sh	1·810	1·80 sh
(0·605)	(0·590)	(0·61)	(0·60)	(0·550)	(0·585)
1·94 sh	—	—	—	—	—
(0·69)					
2·070	2·070	2·075	2·070	2·080	2·080
(0·480)	(0·490)	(0·530)	(0·57)	(0·330)	(0·336)
—	—	—	—	—	—
2·295	2·295	2·300	2·300	2·275	2·295
(0·005)	(0·010)	(0·020)	(0·10)	(0·020)	(0·030)
2·335	2·335	2·340	2·340	2·326	2·340
(0·018)	(0·018)	(0·026)	(0·020)	(0·045)	(0·020)
2·380 br	2·380 br	2·380 br	2·380 br	—	2·380

→ All observations below this rule taken in 1 mm cells, those above, in 1 cm cells.

TABLE 1 (*Continued*)

n-Octanol	*n*-Nonanol	*n*-Decanol	*n*-Dodecanol	*cyclo*Pentanol	*cyclo*Hexanol
(0·040)	(0·040)	(0·040)	(0·030)		(0·042)
2·450	2·450	2·450	2·450	2·42 br	2·440
(0·040)	(0·046)	(0·055)	(0·050)	(0·080)	(0·036)
2·500	2·500	2·500	2·500	—	2·495
(0·080)		(0·085)	(0·100)		(0·050)
2·54	2·540	2·540	—	—	2·540
(0·155)	(0·200)	(0·155)			(0·100)

TABLE 2

NEAR INFRA-RED SPECTRA OF *n*-BROMIDES

Ethyl	*n*-Propyl	*n*-Butyl	*n*-Amyl	*n*-Hexyl	*n*-Heptyl
0·875	0·900	0·910	0·910	0·915	0·910
(0·94)	(0·98)	(0·91)	(*c.* 0·84)	(0·82)	(0·90)
0·885	—	1·02	1·02	1·020	1·020
(0·97)		(0·95)	(*c.* 0·89)	(0·87)	(0·94)
1·156	1·178	1·170	1·161	1·170	1·170
(0·26)	(0·33)	(0·38)	(0·440)	(0·375)	(0·45)
1·185	1·180 sh	1·189	1·181	—	—
(*c.* 0·47)	(0·37)	(0·345)	(0·360)		
—	—	—	1·192 sh	1·197	1·190
			(0·39)	(0·32)	(0·27)
—	1·350 sh	—	—	—	—
	(*c.* 0·67)				
1·360	1·372	1·375 sh	1·372	—	—
(0·420)	(0·46)	(*c.* 0·46)	(0·420)		
1·389	1·388	1·388	—	1·383	1·387
(0·495)	(0·410)	(0·400)		(0·33)	(0·37)
—	1·400	1·400	1·390	1·400 sh	1·400
	(0·530)	(0·450)	(0·475)	(0·36)	(0·39)
1·425	1·428	1·425	1·41 sh	1·42 sh	1·42 sh
(0·690)	(0·575)	(*c.* 0·52)	(*c.* 0·55)	(0·42)	(*c.* 0·45)
1·449	1·451	—	—	—	—
(0·665)	(0·695)				
—	1·461	1·475 sh	—	—	—
	(0·699)	(*c.* 0·7)			
1·500	1·500	1·500 sh	—	*c.* 1·5	*c.* 1·5 sh
(0·900)	(0·880)	(*c.* 0·87)		(*c.*0·85)	(0·91)
1·592	—	—	—	—	—
(0·77)					
1·657	1·670	1·668	1·666	1·670	1·675
(0·004)	(0·02)	(0·040)	(0·060)	(0·100)	(0·12)
c. 1·7	*c.* 1·7	*c.* 1·7	*c.* 1·7	*c.* 1·7	*c.* 1·7
(0·000)	(0·00)	(0·00)	(0·00)	(0·00)	(0·00)
1·740	*c.* 1·76	—	1·74 sh	—	—
(0·070)	(0·015)		(0·01)		
1·770 sh	—	—	—	—	—
(0·38)					
—	1·808	1·8 br.sh	1·8 sh	1·8 br.sh	1·8 br.sh
	(0·18)	(0·12)	(0·09)	(0·070)	(*c.* 0·5)

TABLE 2 (*Continued*)

n-Decyl	*n*-Dodecyl	Cetyl	*n*-Stearyl
0·910 (0·85)	0·915 (0·76)*	0·915 (0·71)*	0·915 (0·02)
1·029 (0·890)	1·020 (0·830)	1·020 (0·85)	1·025 (0·69)
1·170 sh (0·44)	1·155 (0·425)	1·17 (0·46)	1·155 sh (*c.* 0·38)
—	—	—	—
1·193 (0·200)	1·190 (0·155)	1·195 (0·14)	1·190 (0·110)
—	—	—	—
—	1·375 (0·275)	1·375 (0·28)	1·375 (0·240)
1·385 (0·360)	—	—	—
—	—	—	—
1·400 (0·320)	1·395 (0·290)	—	—
—	1·405 sh (0·35)	1·400 (0·295)	1·400 (0·255)
—	—	—	1·41 sh (*c.* 0·31)
—	—	1·450 sh (*c.* 0·36)	—
c. 1·5 br.sh (*c.* 0·82)	*c.* 1·51 br.sh (*c.* 0·74)	1·52 br.sh (0·73)	*c.* 1·56 br.sh (*c.* 0·61)
—	—	—	—
1·675 (0·175)	1·655 (0·120)	1·670 (0·155)	1·660 (0·180)
→			
1·700 (0·290)	1·695 sh (*c.* 0·40)	1·700 (0·25)	*c.* 1·70 (0·000)
1·748 (0·495)	1·75 (0·460)	1·755 (0·43)	*c.* 1·76 (0·000)
1·770 (0·570)	—	1·795 (0·61)	*c.* 1·79 (0·02)
			→

* 1 mm cell.

→ All observations below this rule taken in 1 mm cells, those above in 1 cm cells.

Discussion

Inspection shows that absorptions common to both the alcohols and bromides occur in regions around 1·7, 1·2, and 0·9 μ; these are to be expected and correspond to the second, third, and fourth harmonics of the C—H stretching vibration. Certain rough regularities may be noted: the transmittances at *c.* 0·9 μ are much the same throughout the two classes, but those at *c.* 1·7 μ are notably smaller for the bromides than for the alcohols; among the last-named the transmittances between 1·57 and 1·58 μ, 2·07 and 2·08 μ, and around 1·4 μ after propanol, tend to increase with chain length; the reverse seems to be true for the bands at 1·18–1·19 μ (except for decyl and dodecyl alcohols); for the bromides the transmittances rise for the absorptions 1·155–1·178 μ (except for stearyl bromide) and 1·6–1·7 μ (except for dodecyl bromide) but fall between 1·185 and 1·197 μ and at *c.* 1·4 μ.

Several of these features are displayed on the curves of Figures 1 and 2, in which $\varepsilon_\lambda = (1/c)(\log 1/T)$, measured in 1 cm cells, is shown against n, where c is the number of moles/litre of the pure liquid of transmittance T, and n is the number of methylene groups in $H(CH_2)_nX$, X being —Br or —OH.

It is clear from such curves that empirical relationships could easily be fitted. Alternatively, extinction coefficients at various wavelengths might be correlated in the ways described for hydrocarbons by Rose (1938) or Evans and Hibberd (1951). Either treatment has obvious applications in analysis.

The present appears to be a more extensive survey of the near infra-red region for the *n*-alcohols and *n*-alkyl bromides than any in the literature. Suhrmann and Klein (1941) have previously reported data for ethyl, *n*-propyl, and *n*-butyl, bromides, but the *single* absorptions noted by them at 1·17–1·19 μ are now found to be actually double; the means of the pairs of extinction coefficients so resulting are, however, close to the single values given by Suhrmann and Klein.

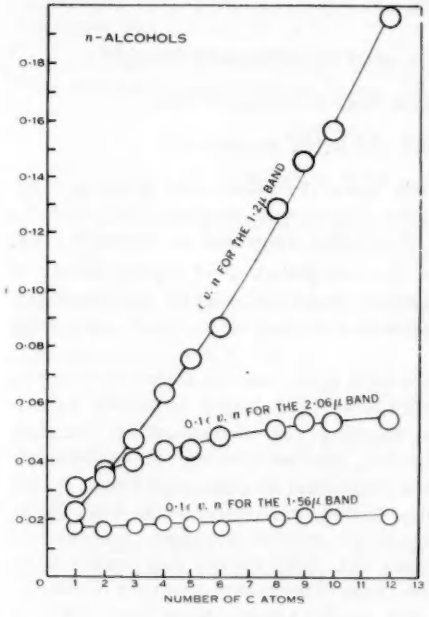


Fig. 1

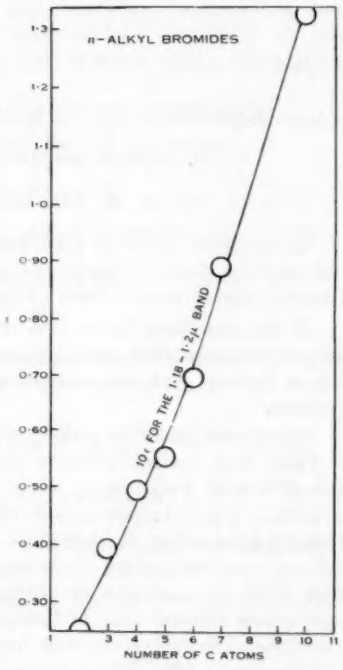


Fig. 2

Fig. 1.—Extinction coefficients of *n*-alcohols.

Fig. 2.—Extinction coefficients of *n*-alkyl bromides. For dodecyl, cetyl, and stearyl bromides 10 ε is 1·9, 2·6, and 3·2 respectively.

Finally, it may be remarked that among the alcohols no monomer-like absorptions, of the type described by Hendricks *et al.* (1936), are uniquely discernible even with the higher members, for which steric inhibition of H-bond association might be expected. It is true that *all* the alcohols absorb between 1·005 and 1·03 μ, but so also do most of the bromides; bands situated between 1·35 and 1·41 μ are common throughout both series. These regions are mentioned since they should contain ν_3^{O-H} and ν_2^{O-H} respectively; evidently to disentangle the effects due to O—H from those due to C—H further work on solutions will be necessary.

References

Evans, A., and Hibberd, R. R. (1951).—*Analyt. Chem.* **23**: 1604.

Hendricks, S. B., Wulf, O. R., Hilbert, G. E., and Liddel, U. (1936).—*J. Amer. Chem. Soc.* **58**: 1991.

Rose, F. W. (1938).—*J. Res. Nat. Bur. Stand.* **20**: 129.

Suhrmann, R., and Klein, P. (1941).—*Z. phys. Chem.* B **50**: 23.

REACTIONS OF TERTIARY BUTYL HYPOCHLORITE*

II. RATE OF REACTION WITH SOME 4–NITROANILINES

By K. H. Pausacker†‡ and J. G. Scroggie†§

It has been shown in Part I of this series (Pausacker and Scroggie 1959) that tertiary butyl hypochlorite (TBH) reacts with 4-nitroaniline, and its *N*-methyl derivatives, to form nuclear-chlorinated compounds in excellent yield.

It has now been found that there is a complete lack of reproducibility of the concentration–time curves when these reactions are followed quantitatively, but, as further work has been discontinued, it is desired to record the results obtained.

Significant induction periods (50–125 min) were observed during the reaction of TBH with 4-nitroaniline in daylight and even longer induction periods (400–1500 min) were noted when the reaction was carried out in complete darkness. The induction period for the dark reaction was reduced considerably (100–250 min) when the solutions were degassed. In one experiment, which had lost 1 per cent. of its oxidizing power in 600 min, only *c.* 60 per cent. of the TBH could be recovered by distillation under reduced pressure. Independent experiments showed that at least 90 per cent. TBH should distil under these conditions. Similar results were obtained from experiments involving *N*-methyl-4-nitroaniline. This would indicate that some oxidant, other than TBH, is present in the reaction mixture and it is proposed that *N*-chloro compounds have been formed. Aliphatic amines can be converted into the corresponding *N*-chloro derivatives with TBH (Bachmann, Cava, and Dreiding 1954; Zimmer and Audrieth 1954) and acetanilide forms *N*-chloroacetanilide with TBH in the presence of sodium hydroxide (Zimmer and Audrieth 1954). However, as TBH is rapidly hydrolysed in dilute alkaline solution to form sodium hypochlorite (Anbar and Dostrovsky 1954), it would appear that sodium hypochlorite could be the chlorinating agent in the latter example.

* Manuscript received April 6, 1959.

† Chemistry Department, University of Melbourne.

‡ Deceased July 20, 1959.

§ This paper represents part of a dissertation submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Melbourne.

The reactions of *N*-methyl-4-nitroaniline and *NN*-dimethyl-4-nitroaniline with TBH also failed to furnish reproducible results and, once again, light-catalysis was noted.

It would appear that these reactions involve free radical intermediates because of (i) light-catalysis, (ii) oxygen-inhibition, and (iii) non-reproducibility of the concentration–time plots. Walling (1957) has shown that other reactions of TBH can display these features. In addition, the simultaneous formation of 4,4′-dinitroazobenzene from 4-nitroaniline, and demethylation products from *N*-methyl-4-nitroaniline and *NN*-dimethyl-4-nitroaniline (see Part I), also suggest free radical intermediates (Pausacker and Scroggie 1954; Mitchell and Pausacker 1957).

It is therefore proposed that the chlorination of 4-nitroaniline and *N*-methyl-4-nitroaniline proceeds, at least in part, by the following mechanism:

$$(CH_3)_3COCl \rightarrow (CH_3)_3CO. + Cl, \quad \ldots\ldots\ldots\ldots \quad (1)$$

$$(CH_3)_3CO. + O_2N.C_6H_4.NHR \rightarrow (CH_3)_3COH + O_2N.C_6H_4.\dot{N}R, \quad \ldots (2)$$

$$O_2N.C_6H_4.\dot{N}R + (CH_3)_3COCl \rightarrow O_2N.C_6H_4.NRCl + (CH_3)_3CO. \ldots (3)$$

(R = H or Me)

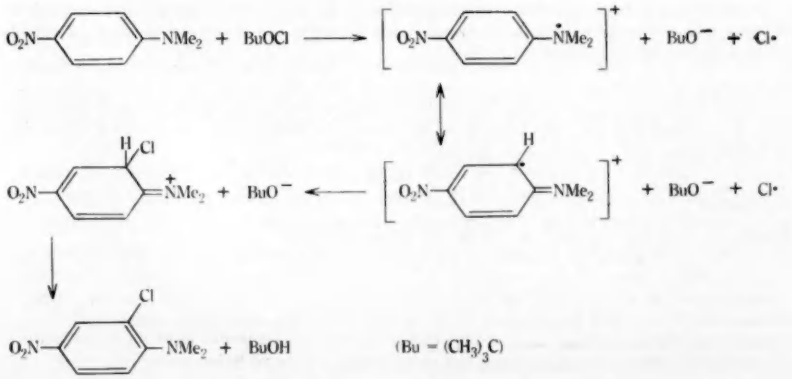
This is similar to the radical chain process proposed for chlorination with TBH by Walling (1957), who has also pointed out that reactions (4) and (5) may be alternative to reaction (2).

$$(CH_3)_3CO. \rightarrow CH_3COCH_3 + .CH_3 \quad \ldots\ldots\ldots \quad (4)$$

$$.CH_3 + O_2N.C_6H_4.NHR \rightarrow CH_4 + O_2N.C_6H_4.\dot{N}R. \quad \ldots\ldots \quad (5)$$

It is then assumed that the *N*-chloro compound formed rearranges by a mechanism similar to that proposed by Ayad *et al.* (1957).

It is also possible to explain the formation of nuclear chlorination products from *NN*-dimethyl-4-nitroaniline as follows:



This mechanism may also be invoked for any direct nuclear chlorination of 4-nitroaniline and *N*-methyl-4-nitroaniline without the intermediate formation of an *N*-chloroamine.

It was thought originally that the reaction may be autocatalytic with respect to the *tert.*-butanol formed. In some cases, notably the reactions of 4-nitroaniline in daylight, the induction period was indeed eliminated when *tert.*-butanol was added. However, it is now considered that this more polar solvent may actually facilitate electrophilic chlorination, at least in part. Ginsburg (1951) has found that 4-methoxybenzaldehyde and TBH yield 3-chloro-4-methoxybenzaldehyde in 90 per cent. acetic acid, but anisoyl chloride is formed in carbon tetrachloride. The first reaction probably proceeds by electrophilic substitution, whereas the second reaction involves a radical mechanism.

Experimental

(i) *Reagents.*—All reagents were prepared and purified as described in Part I.

(ii) *Apparatus and Method of Estimation.*—The amine ($0 \cdot 859$–$4 \cdot 45 \times 10^{-3}$ mol) was accurately weighed into the reaction flask (100 ml) and benzene (*c.* 90–95 ml) was added. The flask and contents were placed in a constant temperature bath at either $20 \cdot 10$ or $30 \cdot 10$ °C and, when temperature equilibrium was obtained, a preheated solution of the appropriate amount of TBH ($0 \cdot 805$–$5 \cdot 25 \times 10^{-3}$ mol) in benzene (5 or 10 ml) was added. The flask was then made up to the mark with preheated benzene and the contents were thoroughly mixed. Aliquot portions (5 or 10 ml) were withdrawn at definite times and added to acidified potassium iodide solution. The liberated iodine was titrated with standardized sodium thiosulphate solution (*c.* $0 \cdot 035$N).

When the rate of reaction was too high, the above procedure was adapted to use with a " Dreischenkelrohr " (Criegee 1932).

The original stock-solution of TBH was maintained under the same conditions as the reacting solutions and was found to be quite stable.

The reactions were initially performed under normal laboratory conditions but, as it is known that TBH is photosensitive (Chattaway and Backeberg 1923), later reactions were carried out in the dark. For this purpose, *all* operations involving TBH were performed in a specially darkened room, the only illumination being provided when necessary by a Wratten Series I photographic safe-light; the reacting solutions were not exposed directly even to this illumination.

The products formed in these rate experiments were occasionally checked qualitatively by evaporation of any residual reaction solution, followed by m.p. and mixed m.p. determinations. In every case, the product identified in this way corresponded with the major product obtained in the appropriate reaction described in Part I.

One of us (J.G.S.) wishes to thank Imperial Chemical Industries of Australia and New Zealand for the award of a Research Fellowship and the University of Melbourne for a Research Grant.

References

Anbar, M., and Dostrovsky, I. (1954).—*J. Chem. Soc.* **1954**: 1094.

Ayad, K. N., Beard, C., Garwood, R. F., and Hickinbottom, W. J. (1957).—*J. Chem. Soc.* **1957**: 2981.

Bachmann, W. E., Cava, M. P., and Dreiding, A. S. (1954).—*J. Amer. Chem. Soc.* **76**: 5554.

Chattaway, F. D., and Backeberg, O. G. (1923).—*J. Chem. Soc.* **123**: 2999.

Criegee, R. (1932).—*Liebigs Ann.* **495**: 219.

Ginsburg, D. (1951).—*J. Amer. Chem. Soc.* **73**: 702.

MITCHELL, J., and PAUSACKER, K. H. (1957).—*Aust. J. Chem.* **10**: 460.
PAUSACKER, K. H., and SCROGGIE, J. G. (1954).—*J. Chem. Soc.* **1954**: 4003.
PAUSACKER, K. H., and SCROGGIE, J. G. (1959).—*Aust. J. Chem.* **12**: 430.
WALLING, C. (1957).—"Free Radicals in Solution." p. 387. (Wiley & Sons: New York.)
ZIMMER, H., and AUDRIETH, L. F. (1954).—*J. Amer. Chem. Soc.* **76**: 3856.

METHYLSTEROIDS*

VI. THE "ISOMERIZATION" OF LANOST-8-EN-3,7,11-TRIONE†

By C. S. BARNES‡ and A. PALMER‡

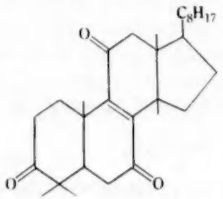
In 1941 when the structure of lanost-8-en-3,7,11-trione (I) was unknown, Wieland and Joost (1941) reported its conversion to an isomer when a solution in 10 per cent. methanolic potassium hydroxide was refluxed for 4 days. The melting point (232° C) of this isomer appeared to us to be too high for a triketone of this series, and moreover, with the structure now known, isomerization appeared impossible. Nevertheless, experiment showed that prolonged refluxing did, as Wieland and Joost reported, give a material with this high melting point, and we have now shown this to be di-(3,7,11-trioxolanost-8-en-2ξyl)methane (II).

In the region around 270 mμ the ultraviolet absorption spectra of I and the new material were identical, showing that the chromophore was unchanged, and that no additional unsaturation had been introduced near a carbonyl group. The infra-red absorption spectra showed no major differences between the two compounds, and in particular no hydroxyl group was present. A molecular weight determination (Rast) showed that in some way two molecules of the triketone had combined, but the data excludes a simple aldol condensation (Wallach 1896).

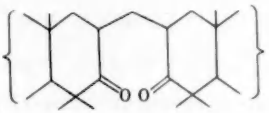
The most likely alternative seemed to be a coupling by a methylene group derived from formaldehyde either present in the solvent or formed from it by base-catalysed hydrogen transfer. This was established by refluxing I with paraformaldehyde in methanolic potassium hydroxide when the product (II) was deposited in 80 per cent. yield within a minute of the addition of paraformaldehyde. In the absence of added paraformaldehyde the yield was less than 50 per cent. even after several hours. The configuration at the 2-position is not assigned, because although it is likely that the substituent is equatorial, the extreme insolubility of the product causes it to precipitate before equilibration could occur. This condensation, which is analogous to the dimedone-formaldehyde reaction, has been studied with simple cyclic ketones by Cologne, Dreux, and Delplace (1954).

* Manuscript received May 8, 1959.
† For Part V of this series see Barnes (1958).
‡ Organic Chemistry Section, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

Lanost-8-en-3-one and cholestan-3-one underwent similar reactions in methanolic alkali, but not with higher alcohols, so that this condensation in methanol is likely to be general for steroidal 3-ketones. The products (type II) were rather unreactive and no satisfactory derivatives were prepared. Oximation gave products which could not be purified and which had nitrogen contents indicating reaction of more than one carbonyl. Pyrolyses also gave no recognizable product.



(I)



(II)

Experimental

(i) *General:* See Part V (Barnes 1958).

(ii) *Condensation of Ketones with Formaldehyde:*

(1) Lanost-8-en-3,7,11-trione (I; 0·5 g) was dissolved in methanol (10 ml) and added to a solution of sodium hydroxide (15 g) in methanol (100 ml). Paraformaldehyde (200 mg) in dilute methanolic sodium hydroxide (10 ml) was added to the refluxing solution. After 30 sec further reflux the deposited crystals (400 mg, 80%) were filtered off and recrystallized from acetone, acetic acid, or *n*-propanol. Only from the last was unsolvated *di-(3,7,11-trioxolanost-8-en-2ξyl)methane* obtained having m.p. 239–240 °C (sealed tube), $[\alpha]_D$ +245°, $\lambda_{max.}$ 270 mμ ε 9,000 (Found: C, 79·5; H, 10·3%; mol. wt., 900±5%. Calc. for $C_{61}H_{92}O_6$: C, 79·5; H, 10·0%; mol. wt., 921).

(2) In a similar way lanost-8-en-3-one reacted with formaldehyde to give *di-(3-oxo-lanost-8-en-2ξyl)methane*, m.p. 233–234 °C (sealed tube), $[\alpha]_D$ ±0°, $\lambda_{max.}$ 290 mμ ε 80 (Found: C, 84·5; H, 11·5%. Calc. for $C_{61}H_{100}O_2$: C, 84·7; H, 11·7%).

(3) In a similar way cholestan-3-one gave *di-(3-oxo-cholestan-2ξyl)methane*, m.p. 259–261 °C (sealed tube), $[\alpha]_D$ +10° (Found: C, 84·0; H, 11·5%. Calc. for $C_{55}H_{92}O_2$: C, 84·1; H, 11·8%).

References

Barnes, C. S. (1958).—*Aust. J. Chem.* **11**: 546.

Cologne, J., Dreux, J., and Delplace, H. (1954).—*C.R. Acad. Sci. Paris* **238**: 1237.

Wallach, O. (1896).—*Ber. dtsch. chem. Ges.* **29**: 2965.

Wieland, H., and Joost, E. (1941).—*Liebigs Ann.* **546**: 103.

INDEX